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Toxicology

New Aspects to This Scientific Conundrum

Edited by Sonia Soloneski and Marcelo L. Larramendy

TOXICOLOGY - NEW ASPECTS TO THIS SCIENTIFIC CONUNDRUM

Edited by **Sonia Soloneski**
and **Marcelo L. Larramendy**

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Meet the editors

Sonia Soloneski is PhD in Natural Sciences and Professor Assistant of Molecular Cell Biology at the Faculty of Natural Sciences and Museum of La Plata, National University of La Plata, Argentina. She became a member of the National Scientific and Technological Research Council (CONICET) of Argentina in Genetic Toxicology field. Presently, she is a member of the Latin American Association of Environmental Mutagenesis, Teratogenesis and Carcinogenesis (ALAMCTA), the Argentinean Society of Toxicology (ATA) and the Argentinean Society of Genetics (SAG). She has authored more than 300 scientific publications in the field, including scientific publications in research papers, reviewed journals and conferences worldwide. She is a referent for issues related to Genetic Toxicology, Mutagenesis and Ecotoxicology field.

Marcelo L. Larramendy, PhD, serves as Professor of Molecular Cell Biology at the School of Natural Sciences and Museum (National University of La Plata, Argentina). He is appointed as Senior Researcher of the National Scientific and Technological Research Council of Argentina. He is also a former member of the Executive Committee of the Latin American Association of Environmental Mutagenesis, Teratogenesis and Carcinogenesis. He is author of more than 470 contributions, including scientific publications, research communications, and conferences worldwide. He has received several national and international awards. Prof. Larramendy is a regular lecturer at the international A. Hollaender Courses organized by the International Association of Environmental Mutagenesis Societies and former guest scientist at National Institutes of Health (USA) and University of Helsinki (Finland). He is expert in Genetic Toxicology and has been referee for more than 30 international scientific journals. He was a member of the International Panel of Experts at the International Agency for Research on Cancer (IARC, WHO, Lyon, France) in 2015 for the evaluation of DDT, 2,4-D and Lindane. Presently, Prof. Dr. Larramendy is Head of the Laboratory of Molecular Cytogenetics and Genotoxicology at the UNLP.

Contents

Preface

Throughout the ages, toxicological science has provided information that has shaped and guided human society. Toxicology, as a distinct scientific discipline, is fairly modern. However, knowledge of poisons and poisoning incidents date back to ancient times. Undertakings as diverse as the healing arts, the industrial military, the agriculture, and the regulation/policy making all have their groundings in the modern toxicology. Toxicology, the science of poisons or the study of the untoward effects of chemicals or physical agents and complex biotoxins on biological systems, has moved over the centuries from the art of food gathering and murder to a highly sophisticated science of mechanisms. The identification and use of toxic substances have occurred throughout our recorded history, and every day, new poisons and toxins are discovered. Humanity has always had the desire to assure its health and safety, but drawing conclusions about harmful chemicals has required both knowledge and learning. Initially, this was done by a trial-and-error system, where substances were tested to see which were safe and which were more hazardous. Then, toxicology advanced significantly throughout the middle ages with an understanding of the relationship between working diseases and exposure to many toxins and historically, this discipline formed the basis of therapeutics and experimental medicine. Paracelsus, a Swiss German physician and alchemist who pioneered the use of chemicals and minerals in medicine and is best known for articulating the concept that *"All things are poison and nothing (is) without poison; only the dose makes that a thing is no poison,"* is today considered the "father" of toxicology. The field of toxicology has come a long way in the centuries since Paracelsus. More than time, research toxicologists have studied the mechanisms of toxicity of a vast range of both naturally occurring and synthetic xenobiotics. More recently, toxicologists have embraced "green chemistry" as the design of chemical and products with properties that minimize or eliminate the negative impacts on humans and on the environment. Modern toxicology has tried to move away from the traditional approach of animal testing and toward a harm-free route of experimentation. Methods have been refined, more multicenter protocols have been performed, and international analysis and exchange of information have increased considerably. The increasing collection and evaluation of biomarkers of exposure and effects is also providing growing opportunities for toxicological science. Actually, the science of toxicology has embraced research in other areas, including genomics, bioinformatics, and more recently, nanotoxicology, which is emerging as a new research discipline. Although live organisms have been used to assess and evaluate potentially toxic products since the eighteenth century, new methodologies continue to be explored, and for some toxic biomarkers, there are no alternatives to animal testing. The ability to detect potential contaminants in water, air and soil can help us identify emerging health threats to living organisms and ecosystems generated by climate change, determine future food sufficiency, and assess the danger of weapons of mass destruction, among others. Rapid detection of potentially harmful physical or chemical xenobiotics is essential for protecting biota in general and human beings in particular.

Although a vast literature is available on toxicology, this book contains solid and important investigations into the diverse chemical hazards encountered in both anthropogenic and natu-

ral environments and provides valuable information about the toxicity of several xenobiotics that can negatively influence the health of humans and ecosystems.

This single volume comprises nine high-quality chapters describing several issues related to the field of toxicology. The first chapter comprises an excellent review, starting with a complete appraisal of the function and structure of the skin, outlining characteristics of cutaneous toxicity, including contact dermatitis, photosensitivity, contact urticaria, chemical-induced acne, pigmentary disturbance, drug rash, hair disturbance, and nail disturbance. This is followed by a second chapter providing information from *in vitro* and *in vivo* studies on the role of hepatic and intestinal multidrug resistance–associated protein 2 in detoxification processes, as well as on their regulation by xenobiotics, at the transcriptional and post-transcriptional levels. The third chapter presents an update describing the chemical and physical properties of nanoparticles and their characterization for proper toxicological evaluation related to exposure, environmental fate and transport, and ecological and genotoxic effects, with a focus on the risk of these in aquatic environment biota. The fourth chapter provides information about embryotoxic and teratogenic effects induced by maltodextrin-coated cadmium sulfide quantum dots in chicken embryos and fetuses. The fifth chapter provides an overview of applications of zinc oxide nanoparticles followed by a brief outline of methods of synthesis and characterization and the current knowledge on zinc oxide nanoparticle interactions with fish and their potential implications for human health. The sixth chapter depicts the impacts of microplastics in aquatic environments and their toxicological implications for fish populations. The seventh chapter is an interesting overview of several types of ecotoxicological biomarkers, presenting a set of examples about employed species, advantages and disadvantages of different types of toxicity testing, and the use of exposed natural ecosystems or man-made analogue systems as the more environmentally realistic approach for ecotoxicological testing. The eighth chapter presents a review about cyanogenic glycosides in specific plant foods, the health implications of consuming cyanogenic plants, and the effects of various processing methods on cyanogenic glycosides, with updated information gathered from the published reports on cyanogenic glycosides. The book ends with a chapter describing the possible toxicities of herbal medicines and their different alternatives, creating a sense of awareness in people to avoid the use of herbal medicines without prescription and to motivate different underdeveloped and developing countries to promptly make regulations for herbal medicines.

The editors of *Toxicology - New Aspects to This Scientific Conundrum* are enormously grateful to all the contributing authors for sharing their knowledge and insight into this book project. They have made an extensive effort to arrange the information included in every chapter. This book is designed to provide an overview on the different toxicants and their effects on living organisms, including humans. The publication of this book is of high importance for those researchers, scientists, engineers, geneticists, pharmacologists, physicians, and veterinarians, as well as teachers and advanced-level students, who make use of these different investigations to understand both basic and applied toxic aspects of known and new xenobiotics and to guide them in the future investigations.

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Chapter 1

Drug-Induced Cutaneous Toxicity

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Additional information is available at the end of the chapter

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Abstract

The skin is the largest organ in the body and is continually exposed to external stimuli, such as chemical and environmental substances. Cutaneous toxicity can be broadly classified according to the mechanism of onset, namely: contact dermatitis, i.e., damage resulting from contact with a substance (irritant dermatitis, allergic contact dermatitis, chemical burns); photosensitivity, i.e., caused by combined effects of a substance and ultraviolet light (phototoxic dermatitis, photoallergic contact dermatitis); contact urticaria; chemical-induced acne; pigmentary disturbance; drug rash; hair disturbance; nail disturbance; or tumor-induced. This review outlines the function and structure of the skin, outlining characteristics of these types of cutaneous toxicity. In recent years, advances have been made in the development of pharmaceutical products targeting specific molecules or genes and nanotechnology-based pharmaceutical products, raising concerns about the onset of toxicity by novel mechanisms involving new pharmaceutical products. Therefore, it is important to understand the basic toxicity-related changes described herein.

Keywords: cutaneous toxicity, drugs, chemicals, toxicity studies

1. Introduction

Cutaneous adverse drug or chemical reactions in patients are not common. Among hospitalized patients, the incidence of adverse drug reactions concerning the skin ranges from 1% to 3%; however, the actual prevalence is much higher, as many mild forms of cutaneous adverse reactions are not reported [1]. We are constantly exposed to external stimuli, such as chemical and environmental substances, resulting in various skin symptoms. This article focuses on (1)

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the function and structure of the skin and (2) characteristics of cutaneous toxicity of pharmaceutical products and chemical substances in humans and animals.

2. Function and structure of the skin

Skin is the largest organ of the body, covering the surface and accounting for approximately 15–20% of body mass. In addition to its constant barrier role, protecting the living body against external stimuli, skin is important for maintaining the body health (e.g., through regulation of body temperature, storage of fluids and electrolytes, and the synthesis of vitamin D) and also acts as an important component of the immune system. As a sensory organ, the skin can sense pain, touch, pressure, and temperature [2]. Histologically, the skin consists of the epidermis, dermis, and subcutaneous tissue. The epidermis is formed by keratinized squamous epithelia, stratified from the surface into the cornified layer, clear layer (only on the palms and soles), granular layer, spinous layer, and basal layer [3]. The epidermis also contains antigen-presenting Langerhans cells (mainly in the spinous layer); melanocytes (mainly in the basal layer), which produce melanin to protect epidermal cells against damage induced by ultraviolet light; and Merkel cells (mainly on the palms and soles), which are neuroendocrine cells. The dermis is composed of fibrous connective tissue with elastic and reticular fibers intermingled with collagen bundles, containing mast cells that are involved in allergic reactions, sweat glands, sebaceous glands, hair follicles, blood vessels, lymphatic vessels, and nerve fibers. Subcutaneous tissue is composed of loose connective tissue and subcutaneous adipose tissue. Adipose tissue is especially prominent in the footpads where it functions as a "shock absorber" and as an insulating layer [4]. Skin appendages are skin-associated structures that serve a particular function, including sensation, contractility, lubrication, and heat loss. They contain hairs (sensation, heat loss, filter for breathing, protection), sebaceous glands (secrete sebum onto hair follicles, which oils the hair), sweat glands (can produce sweat secreted with strong odor (apocrine) or with a faint odor (eccrine), and nails (protection). Hair growth occurs in three stages: anagen (growth phase), catagen (involution period), and telogen (resting phase during which hair shedding occurs) [5, 6]. The rate of hair growth and duration of the growth cycle vary in different areas of the body and are influenced by sex hormones and growth factors. Sebaceous glands are most often associated with hair follicles and produce sebum by holocrine secretion. Zymbal's gland is a specialized sebaceous gland in rodents located at the base of the external ear canal; the gland cells contain cytochrome P450 isoenzyme and peroxidases and are capable of chemical metabolism [7, 8]. Apocrine glands are distributed throughout the skin of most laboratory animals, whereas in humans they are located in axillary, pubic, and perianal areas, while they are only present in the plantar areas in rodents. There are a number of specialized apocrine glands, such as the anal sac gland of dogs, the ceruminous glands of the external ear canal, and the glands of Moll in the eyelids. Eccrine glands are found throughout the body in humans; however, these glands are limited to the footpads of carnivores and rodents.

In preclinical studies, cutaneous toxicity is rarely encountered, except in cutaneous application, intradermal administration, and subcutaneous administration. Cutaneous toxicity primarily

involves either a direct local inflammatory reaction to the drug without involvement of an immunological mechanism or an indirect inflammatory reaction associated with a systemic manifestation [6]. In cutaneous application studies, both epidermis and skin appendages are important factors in transdermal drug absorption [9]. Experimental animals such as guinea pigs, monkeys, and swine exhibit similar absorption characteristics to humans [5]. Of all laboratory animals, swine skin is most structurally comparable to human skin [4]. Swine and humans have comparable stratum corneum, epidermal thickness, and hair follicle density, as well as similar chemical composition of the stratum corneum. Rodents have much thinner skin (especially the epidermis) with greater permeation compared to humans [4]. In general, skin is thicker over the dorsal and lateral surfaces and thinnest on the ventral and medial surfaces. Areas of skin that contact the ground, such as footpad and heels, have the thickest epidermis (**Figure 1**). The extent of transdermal drug absorption differs according to skin location. Sites in order of favorable absorption, due to the skin thickness, are the abdomen, forehead, palms, and soles of feet [5]. It should be noted that skin thickness varies considerably during the hair cycle (**Figure 2**). Skin thickness during the anagen stage is thickest and is thinnest during the catagen stage in rodents and rabbits. If the skin is damaged, the biological protective barrier function decreases, leading to a significant increase in drug absorption, which results in intensified systemic toxicity [10]. Microsomal enzymes in keratinocytes are capable of metabolizing topically applied chemicals, thus rendering them inactive or active. Dimethylbenz(a)anthracene (DMBA) becomes a potent skin carcinogen after metabolic activation by keratinocytes [11].

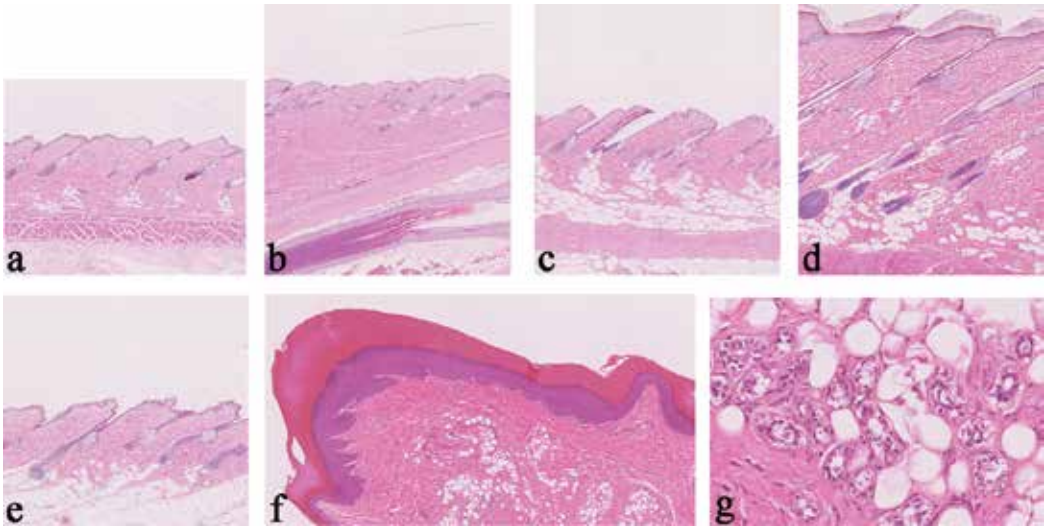

Figure 1. Comparative histology of different skin locations in rats. (a) Scalp region at the vertex. (b) Nose region. (c) Inguinal region. (d) Back region. (e) Abdominal region. (f) Footpad region. (g) Eccrine sweat gland in the footpad. Note that scalp, inguinal, and abdominal skin are thin. In contrast, back and footpad skin are thicker. Footpad skin is the thickest, especially the stratum corneum and epidermis. Eccrine sweat glands are located only in the footpad of rodents; however, these glands are found throughout the human body.

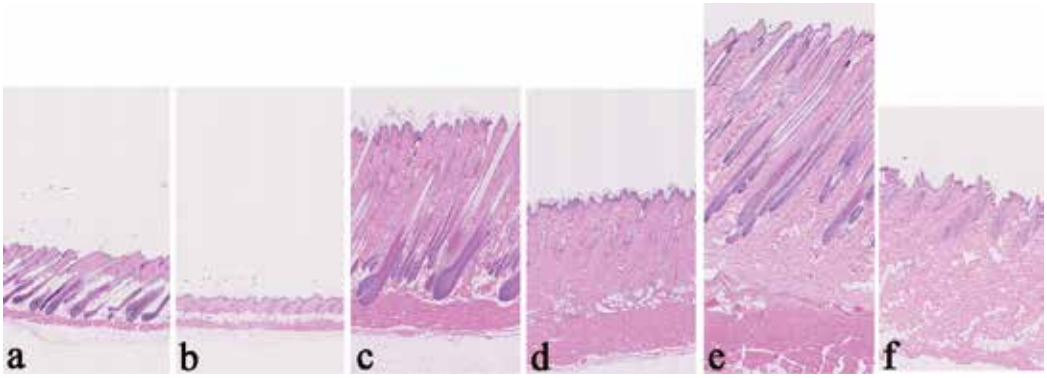

Figure 2. Comparative histology of back skin during different hair cycle stages. (a) Anagen stage in the mouse. (b) Catagen stage in the mouse. (c) Anagen stage in the rat. (d) Catagen stage in the rat. (e) Anagen stage in the rabbit. (f) Catagen stage in the rabbit. Note that skin thickness is thickest at the anagen stage and thinnest at the catagen stage in rodents.

3. Types of cutaneous toxicity (Table 1)

Cutaneous toxicity can be classified according to the mechanism of onset into the following: (1) contact dermatitis, i.e., damage resulting from contact of the skin with a drug (irritant dermatitis, allergic contact dermatitis, and chemical burns); (2) photosensitivity, caused by the combined effect of a chemical substance and ultraviolet light (phototoxic dermatitis and photoallergic contact dermatitis); (3) contact urticaria; (4) chemical-induced acne; (5) pigmentary disturbance; (6) drug rash; (7) hair disturbance; (8) nail disturbance; and (9) tumor-induced. Cutaneous toxicity can also be classified according to the route of exposure, i.e., either due to systemic effects or local irritation of the skin (local toxicity) [5, 6].

3.1. Contact dermatitis

Contact dermatitis is skin inflammation occurring as a result of direct contact of the skin with a drug that can be classified into the following three types, according to the mechanism of onset.

3.1.1. Irritant dermatitis

Irritant dermatitis is an inflammatory change caused by direct irritation of the skin that can be either acute or cumulative. Activation of mast cells, complement or prostaglandin synthesis results in reversible damage to the skin, observed as irritation within 4 hours following topical application of the chemicals. Irritant dermatitis is characterized by inflammatory cell infiltration, acanthosis, epidermal hyperkeratosis, and hyperplasia associated with other epidermal changes such as erosion/ulcer, necrosis, or vesicle formation [11]. Irritant dermatitis depends on the severity of the irritants and duration of their exposures [5, 6] (**Figure 3**). If the damage to the skin is irreversible, the lesion is clinically referred to as corrosion, which is characterized by full thickness necrosis of the epidermis penetrating into the underlying dermis [11]. In preclinical

studies of topical application agents, skin irritation testing is conducted using rabbits or guinea pigs to evaluate drug-induced irritation using the Draize method (with a 5-grade score based on macroscopic assessment of the severity of erythema, crusting, and edema) (**Figure 4** and **Table 2**). The Draize test consists of application of the chemical to the test site on shaved dorsal skin. The test sites undergo gross evaluation at 6, 24, and 72 postapplication.

Classification	Type	Definition and characteristics
A. Classification according to the route of exposure to the drug	Cutaneous toxicity due to systemic effect Local irritation of skin (local toxicity)	
B. Classification according to the mechanism of onset		
1. Contact dermatitis	Irritant dermatitis	Skin inflammation occurring as a result of direct contact of the skin with a drug, without involvement of an immune mechanism
	Allergic dermatitis	Skin inflammation upon re-exposure to a drug that had been previously administered and bound as a hapten to a protein in the skin to become immunogenic (type IV allergic reaction)
2. Photosensitivity	Phototoxic dermatitis	A condition caused by a drug with covalent binding as a result of a photochemical reaction with ultraviolet light
	Photoallergic dermatitis	Skin inflammation upon re-exposure to a previously administered drug that absorbed ultraviolet light and was transformed to act as a hapten to bind with a protein in the skin to become immunogenic (type IV allergic reaction)
3. Contact urticaria		Acute erythema with involvement of histamine release from mast cells (increased vascular permeability), occurring soon after contact with the drug
4. Chemical acne		Inflammation of hair follicles due to excessive keratin and sebum in hair follicles
5. Pigmentary disturbance	Hyperpigmentation	A condition occurring in association with increased melanin production due to activation of melanocytes, hemosiderin deposition due to hemorrhage, or deposition of the drug itself
	Hypopigmentation	A condition occurring in association with loss of melanin or selective damage to melanocytes
6. Drug rash (cutaneous reaction)	Toxic epidermal necrolysis, oculomucocutaneous syndrome	The mechanism remains unknown, although an allergic reaction has been speculated. Reported for greater than 1100 drugs, including sulfa drugs
7. Hair disturbance	Alopecia	A condition due to drugs with an androgenic effect acting on hair follicles to shorten the hair cycle, or drugs with an antimitotic effect inducing atrophy of hair follicles and prolongation of the resting phase of the hair cycle
	Hypertrichosis	A condition due to prolongation of the anagen phase of hair follicles induced by certain immunosuppressants, antihypertensives (minoxidil), or drugs for benign prostatic hyperplasia (finasteride)
8. Nail disturbance	Nail transverse ridges, onycholysis, discoloration	A condition arising from damage to the nail matrix cells due to drugs with an antimitotic effect or deposition of the drugs themselves
9. Tumors		

This table has been modified from [5].

Table 1. Classification of drug-induced cutaneous toxicity.

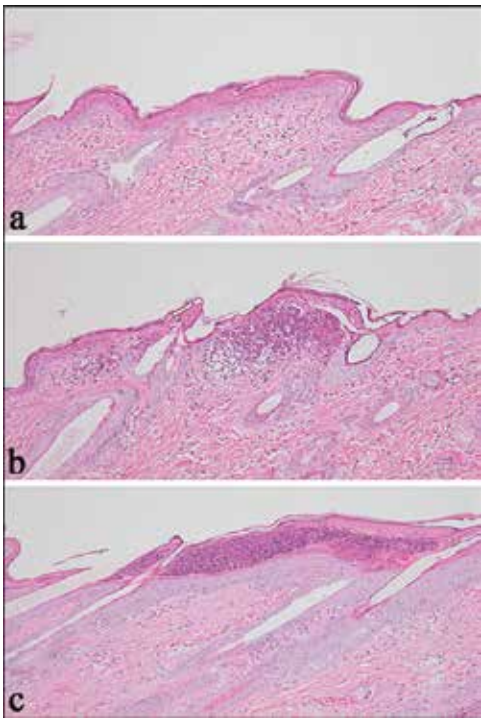

Figure 3. Sequential stages of inflammatory changes in irritant contact dermatitis following a single exposure to sodium lauryl sulfate (SLS) in the guinea pig. (a) Epidermal necrosis and slight infiltration of neutrophils in dermis are observed 24 hours after exposure. (b) Epidermal necrosis and severe infiltration of neutrophils in epidermis and dermis are observed 24 hours after exposure. (c) Epidermal abscess (pustule) and acanthosis (epidermal regeneration) are observed 48 hours after exposure. Owing to its emulsifying properties, SLS is an anionic surfactant used in many hygienic and cleaning products, including shampoos, toothpastes, and shaving foams.

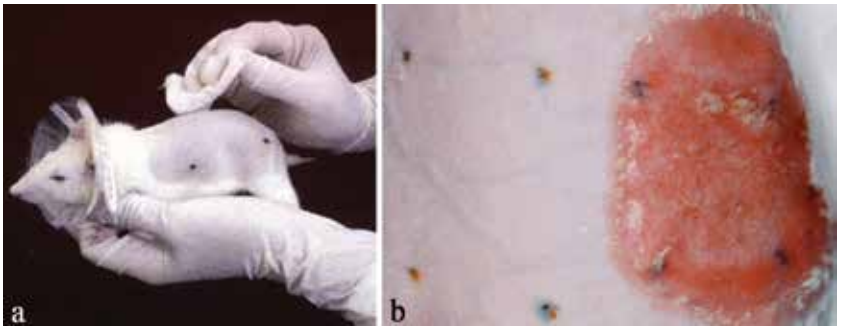

Figure 4. Macroscopic photos from a cumulative dermal irritation study in animals. (a) After the hair on the back of the rat is shaved, the drug is continually applied to the same area. The rat wears the Elizabethan collar to prevent the animal from biting or licking the exposure site. (b) Cumulative dermal irritation study in a rabbit (left: vehicle application, right: drug application). The site of drug application is observed with erythema, redness, swelling, and moistness. The change spreads beyond the site of application, indicating a strong irritant property of the drug.

Skin reaction	Grading value
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate-to-severe erythema	3
Severe erythema to slight eschar formation	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (raised edges of area well defined)	2
Moderate edema (raised more than 1 mm)	3
Severe edema (raised more than a mm and extending beyond the area of exposure)	4

Eschar is scab or crust formation.
This table has been modified from [4].

Table 2. Skin irritation test (Draize scale).

3.1.2. Allergic contact dermatitis

Allergic contact dermatitis is a condition caused by a delayed (type IV) allergic reaction. A low molecular weight drug binds as hapten to a protein in the body to act as a complete antigen. Characteristically, inflammation is induced approximately 12 hours following recontact of a sensitized animal with the drug. Known sensitizing substances include preservatives contained in topical application agents, nickel sulfate, potassium dichromate, neomycin, aroma chemicals, formaldehyde, rubber/latex medical supplies, and plants (e.g., rhus lacquer).

3.1.3. Chemical burns

Chemical burns are an injury caused by a chemical substance that is extremely corrosive or irritating (e.g., strong acid or strong alkali), often involving itching and/or ulceration due to local coagulative necrosis (**Figure 5**). No currently available pharmaceutical products cause this type of injury. Accidental exposure to skin or oral ingestion of these chemicals represents a pediatric emergency problem and these chemicals have a history of being common agents used for suicide [12, 13]. Cement burn is well known in the developed world. The majority of patients are either workers in the construction industry or do-it-yourself enthusiasts, commonly kneeling or standing in cement. The mechanism of injury is a combination of the effects of cement alkalinity and mechanical abrasion. Besides denaturing protein, alkalis saponify fat-producing liquefactive necrosis [14].

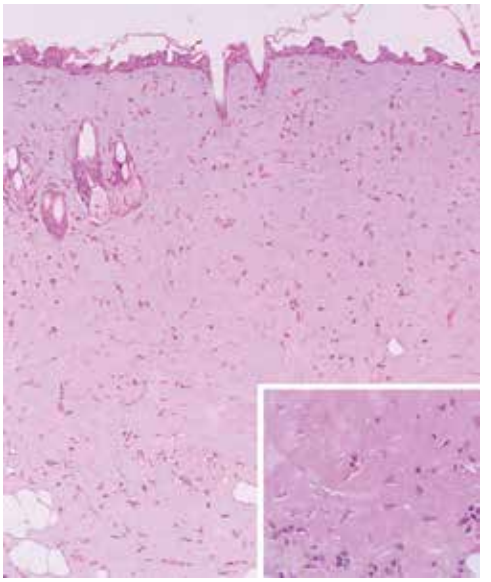

Figure 5. Sodium hydroxide-induced burn in the back skin of a rat. Severe coagulative necrosis is observed in all cutaneous layers. Insert is a higher magnification image of the same photo.

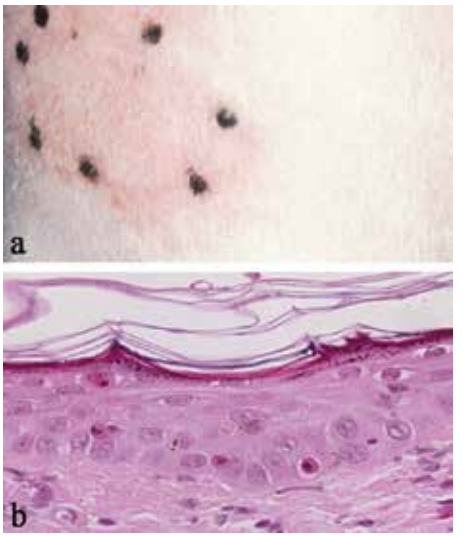

Figure 6. Macroscopic photo from a phototoxic study of 8-methoxypsoralen in guinea pigs. After the hair on the back of the guinea pig was shaved, the drug was applied to the same area and irradiated with ultraviolet light, and the reaction was subsequently evaluated. (a) The site on the left was irradiated with ultraviolet light (UVA) after drug application, while the site on the right was not irradiation after drug application. Erythema is observed at the site with ultraviolet light irradiation. This reaction to 8-methoxypsoralen with ultraviolet light has been utilized in ultraviolet light therapy (PUVA) for psoriasis in humans. (b) Apoptotic epidermal cells (sunburn cells) are observed at the site with ultraviolet light irradiation.

3.2. Photosensitive dermatitis

3.2.1. Photosensitivity

Photosensitive dermatitis (photosensitivity) is a general term that refers to skin inflammation caused by the combined effect of a drug and light. It can be classified into two types, either with or without involvement of an immunological mechanism. Numerous systemic and topical drugs, aroma chemicals, plants, and cosmetics have been reported to induce this condition. Some examples of photosensitizing drugs are phenothiazine, tetracyclines, sulfonamides, chlorpromazine, nalidixic acid, and fluorocoumarins (psoralens).

3.2.2. Phototoxic dermatitis

Phototoxic dermatitis is skin damage caused by a drug that is sensitive to light (ultraviolet light), not by the drug alone, but after absorption of photon energy, without involvement of an immune mechanism. Free radicals and peroxidative injuries have been reported to be involved in this reaction. In preclinical studies of topical application agents, phototoxicity testing is conducted using guinea pigs for evaluation of drug phototoxicity (**Figure 6**).

3.2.3. Photoallergic contact dermatitis

Photoallergic contact dermatitis is a condition caused by a delayed (type IV) allergic reaction. A drug sensitive to light (ultraviolet light) absorbs photon energy and is transformed into a substance (i.e., hapten) that combines with a protein in the body to act as a complete antigen. Characteristically, inflammation is induced approximately 12 hours following recontact of a sensitized animal with the drug.

3.3. Contact urticaria

Contact urticaria is acute redness or rash that occurs within several minutes to one hour following exposure to a drug. It can be caused by a direct effect of the drug on vascular walls, by an indirect effect on vascular walls via histamine release from mast cells (without involvement of an immune mechanism), or by an IgE-mediated immediate (type I) allergic reaction with involvement of an immune mechanism. For immune contact urticaria, known conditions include systemic reactions to penicillin or food, as well as urticaria due to natural rubber products (latex allergy), but it is generally difficult to reproduce such conditions in preclinical studies using experimental animals.

3.4. Chemical-induced acne

Chemical-induced acne is a disease of hair follicles caused by a chemical substance and is characterized by keratin plugs in hair follicles due to excessive proliferation of keratinocytes in hair follicles (comedo), sebum retention and inflammation [11]. Known examples of chemical-induced acne include occupational skin disorders of oil acne, caused by frequent exposure of the skin to cutting oils, as well as chloracne, induced by dioxins such as TCDD and PCB [15]. Clinically, the lesions are located around the eyes, ears, back, and genitalia; and

other symptoms include hyperpigmentation, conjunctivitis, and ocular discharge. A notorious event occurred when Ukraine President Viktor Yushchenko was stricken with facial chloracne resulting from deliberate poisoning with TCDD during his presidential campaign [16].

3.5. Pigmentary disturbance

Pigmentary disturbance is only observed in animals with scarce hair or animals that have been shaved, thus is difficult to detect in preclinical studies. Altered pigmentation is a condition that sometimes follows skin inflammation and is characterized histopathologically by an increase or decrease in the number of melanocytes as well as melanin production. Hyperpigmentation can occur in association with increased melanin production due to drug-induced activation of melanocytes, hemosiderin deposition due to hemorrhage, or deposition of a heavy metal or drug itself (**Figures 7** and **8**). Melanin production is increased by busulfan, cyclophosphamide, long-term high-dose ACTH, and inorganic arsenic. In addition, chlorpromazine or minocycline can form a complex with melanin or hemosiderin with deposition in the skin, leading to blue-gray discoloration of the skin. In contrast, hypopigmentation results from loss of melanin due to damage to melanocytes. Depigmenting agents such as phenols, catechols, and hydroquinone have a similar structure to tyrosine, thus can inhibit melanin synthesis and induce hypopigmentation (**Figure 8**). Recently, an unexpected outbreak of patients with leukoderma occurred in Japan with use of brightening/lightening cosmetics containing rhododendrol, which is a competitive tyrosinase inhibitor, thereby inhibiting melanin synthesis [17]. This type of leukoderma is induced by not only apoptosis of melanocytes but also subsequent immune reactions with CD8-positive T cell infiltration toward melanocytes [18, 19].

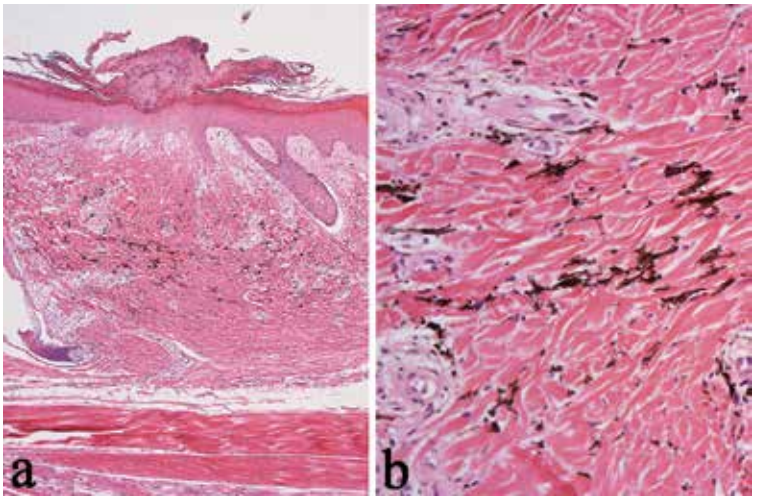

Figure 7. Hyperpigmentation due to melanin deposition in dermis at a drug injection site in the monkey. (a) Crust and epidermal hyperplasia are observed in the epidermis, and black pigment is observed in the dermis. (b) High power field of (a). Note that the black pigment is scattered throughout the dermis.

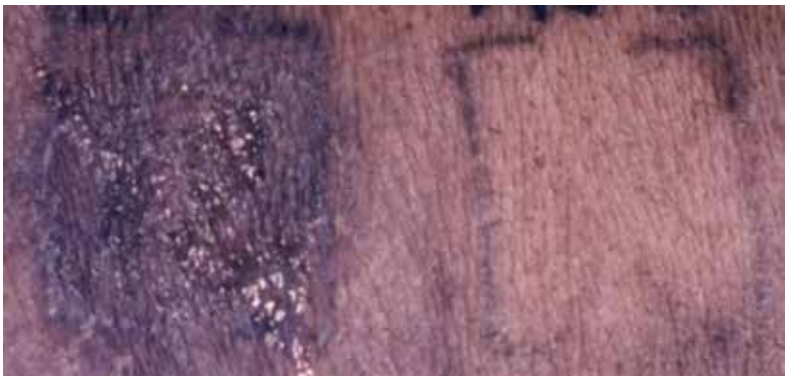

Figure 8. Macroscopic photo from a dermal application study of two drugs in mini-pigs (dark Yucatan pigs). The normal skin of Yucatan pigs appears black, because the skin contains a large amount of melanin pigment. With one drug applied to the left regions of the image, darkening of the skin is shown compared to the normal portion of skin, indicating excessive pigmentation due to drug application. With another drug applied to the right side of the image, lightening of the skin is shown with decreased pigmentation.

3.6. Drug rash (cutaneous reaction)

Drug rash (cutaneous reaction) is the most common adverse drug reaction reported to occur with antibiotics. The most serious forms of drug rash are toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome, which are known to occur with the use of various drugs, including penicillin derivative or cephem derivative antibiotics, antipyretic analgesics (particularly NSAIDs), allopurinol, amine antiepileptic drugs (phenytoin and carbamazepine), and sulfa drugs. The Ministry of Health, Labour and Welfare of Japan announced that of 110,023 cases of adverse drug reactions reported from 2005 to 2009, approximately 2.2% of the cases (2370) were toxic epidermal necrolysis or Steven-Johnson syndrome [20]. Although the mechanism of onset remains unknown in many instances, a type III allergic reaction is often speculated.

Many new antitumor drugs with specific molecular targets have been approved in recent years (the so-called "targeted therapies"), and their adverse effects are highly specific with respect to the skin. Cutaneous reactions to these therapies are among the most frequently observed and, when severe or protracted, can result in significant morbidity, requiring dose modification or drug discontinuation [21, 22]. Hyperplastic changes of the epidermis can be attributed to numerous causes, including response to stimulation from growth factors, such as epidermal growth factor (EGF). The repeated administration of EGF to cynomolgus monkeys results in cutaneous desquamation and epidermal hyperplasia [23]. Epidermal growth factor receptor (EGFR), multikinase, c-Kit, BRAF, or MEK inhibitors induce papulopustular rash, maculopapular rash, and hand-foot syndrome in humans [24, 25]. EGFR inhibitor-induced lesions are associated with the inhibition of EGFR in undifferentiated, proliferating keratinocytes in the basal and suprabasal layers of the epidermis [26]. Other inhibitor-related rashes appear to be associated with the inhibition of vascular endothelial growth factor (VEGF) receptors in the skin [24].

3.7. Hair disturbance

Many drugs induce hair disorders, such as hair loss, stimulated hair growth, or, more rarely, changes in the hair shape and color [27]. Hair loss (hypotrichosis or alopecia) is a common problem that affects approximately 60 million men, women, and children in the United States, with a total cost for medical consultation and treatment of US$1.3 billion per year [28]. The onset of alopecia (toxic alopecia) often depends on the hair cycle at the time of drug administration. Drugs with an androgenic effect can cause alopecia by acting on the resting phase of the hair cycle to shorten the cycle. In addition, drugs with an antimitotic effect (e.g., anticancer drugs) or irradiation can cause alopecia by inducing apoptosis of hair follicles during the anagen phase of the hair follicle, thereby causing atrophy of hair follicles and prolongation of the resting phase of the hair cycle (chemotherapy or radiation-induced follicular dystrophy) [29, 30] (**Figure 9**). Hypertrichosis refers to drug-induced promotion of hair growth or induction of the anagen phase [31] **Figure 10**, and has been reported in organ transplant recipients and animal models treated with cyclosporine [32], as well as an antihypertensive (minoxidil) and a drug for treating benign prostatic hyperplasia (finasteride) [33]. Minoxidil and finasteride have been approved for clinical use as drugs to stimulate hair growth [34]. Hypertrichosis is observed as an increase in the length, thickness, and number of eyelashes in glaucoma patients treated with prostaglandin F2α agonists [35]. Bimatoprost has been used as a therapy for eyelash insufficiency or as eyelash restorer. As in the cases of the pigmentary disturbances described above, these changes can only be observed in animals with scarce hair or animals with shaved hair and are thus difficult to detect in typical preclinical studies.

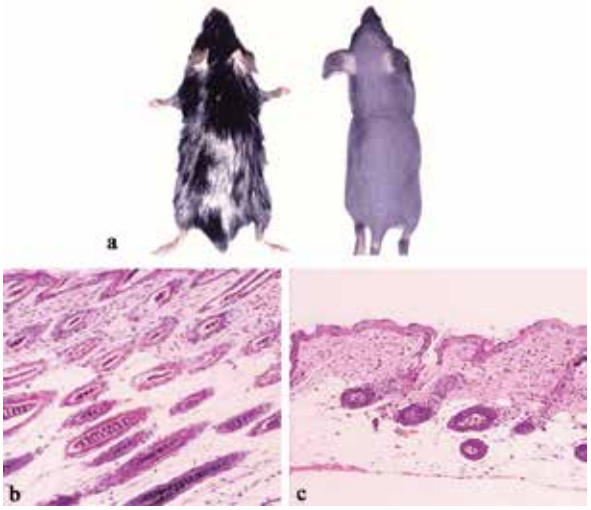

Figure 9. *N*-Methyl-*N*-nitrosourea (MNU)-induced follicular dystrophy in C57BL mice. (a) Systemic hair loss and whitening skin color are observed in the mouse treated with MNU (right side), compared to the control mouse with abundant hair (left side). MNU is an alkylating agent used as an antimitotic chemical, and induces apoptosis of hair follicles during the anagen phase of the hair follicle causing atrophy of hair follicles and prolongation of the resting phase of the hair cycle. (b) Anagen stage of hair cycle in a control mouse. (c) Catagen stage of hair cycle (follicular dystrophy) in a MNU-treated mouse. (b) and (c) are at the same magnification.

Figure 10. Immunosuppressant drug-induced hypertrichosis in a mouse follicular dystrophy model. (a) Compared to *N*-methyl-*N*-nitrosourea (MNU)-induced follicular dystrophy in the treated mouse (left side), the mouse treated with the immunosuppressant drug after MNU exposure has abundant body hair (right side) (b) MNU-induced follicular dystrophy is characterized by a prolonged catagen stage of hair cycle and thinness of the back skin. (b) and (c) are at the same magnification. (c) The hair cycle in the mouse treated with the drug after MNU exposure was found to be in the anagen stage with thickening of the back skin. (b) and (c) are at the same magnification.

3.8. Nail disturbance

Nail changes that reflect a previous general condition are a barometer of health that can be used to predict the presence or absence of an abnormality several weeks before its presentation [6]. Transverse ridges (Beau's line), washboard nail plates, and onycholysis are known to occur with use of metoprolol, retinoids, anticancer drugs, or irradiation [5]. Adverse effects of targeted molecular therapies, such as EGFR inhibitors, are also highly specific with respect to nails in human patients [22, 36]. In addition, yellow nail discoloration is known to occur with penicillamine, and black nail discoloration with Futraful (tegafur), anticancer drugs, or gold drugs. Administration of nucleoside analogs to dogs results in nail loss and footpad erosions with associated radiomimetic defects in the stratum germinativum [4]. In general, onycholysis can be induced by anticancer drugs or irradiation in experimental animals, while other changes are difficult to detect in preclinical studies.

3.9. Skin carcinogenesis

Some photoirritants, such as 8-methoxypsoralen, have been associated with UV-induced skin carcinogenesis. Treatment of psoriasis by photochemotherapy (PUVA) with oral methoxsalen, a psoralen, in conjunction with UVA radiation, is associated with an increased risk of irregular

pigmented skin lesions, squamous cell carcinoma, and malignant melanoma [37, 38]. Chemically induced skin tumors have been associated with numerous topically applied and systemically administered compounds in rodents; however, there appear to be few clinically used drugs that are suspected of being involved in skin carcinogenesis in humans. Rodent models of skin carcinogenesis are widely used for studies of carcinogenic mechanisms and the evaluation of carcinogenesis associated with chemical substances. Huff et al. performed a retrospective investigation of carcinogenicity tests on 379 chemical compounds conducted by the US National Toxicology Program (NTP) and reported that increased skin carcinogenesis was observed with 19 chemical compounds [39] (**Table 3**). Currently used methods used to determine skin carcinogenesis of drugs/chemical substances or methods to clarify carcinogenic mechanisms include: 2-year dermal application carcinogenicity studies (**Figure 11**); DMBA/TPA two-stage skin carcinogenesis models using 12-O-tetradecanoylphorbol-13-acetate (TPA) and 7,12-dimethylbenzanthracene (DMBA) (DMBA as an initiator, TPA as a promoter) [40]; studies in Tg.AC transgenic mice with expression of the v-Ha-ras gene in the epidermis [41]; and studies in SENCAR (SENsitivity to CARcinogen) mice [42]. Two-stage skin carcinogenesis models using metallothionein-I/II knockout mice have shown significant increases in skin carcinogenesis, thereby indicating an important role of metallothionein as an inhibitory factor of carcinogenesis in skin [43]. p53 is a protein that causes cell cycle arrest, apoptosis, or senescence that is crucial in the process of tumor suppression in several cell types [44]. In the

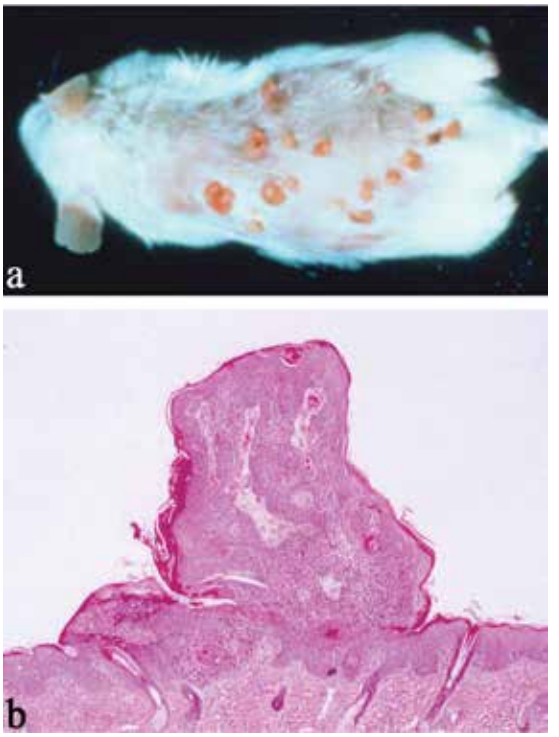

Figure 11. A 2-year dermal application study of a drug in CD1 mice. (a) Macroscopically, multiple reddish skin tumors are observed on the back. (b) Histological findings are consistent with squamous cell papilloma.

DMBA/TPA two-stage skin carcinogenesis model, the absence of p53 in stratified epithelia leads to the appearance of a higher number of tumors that grow faster and become malignant more frequently than tumors arising in mice with the wild type p53 genotype [45]. The carcinogenic risk of a chemical after topical application is traditionally investigated in rats; however, in recent years, Tg.AC mice have become a popular alternative. The skin of Tg.AC mice is genetically initiated, thus the induction of epidermal papilloma in response to dermal or oral exposure to a chemical agent acts as a reporter phenotype for the carcinogenicity of the test chemical [11, 46]. The SENCAR mouse is an outbred strain (not genetically engineered) that was selected specifically for increased skin tumor multiplicity and decreased tumor latency in response to known dermal carcinogens [41]. A recent report described a possible animal model for human keratoacanthoma involving a single intraperitoneal injection of 50 or 75 mg/kg *N*-methyl-*N*-nitrosourea in male Sprague-Dawley rats at 4 weeks of age [47] (**Figure 12**). Keratoacanthoma is a benign tumor believed to arise from the epithelium of hair follicles [48]. Peroxisome proliferator-activated receptor agonists have been associated with the development of hemangiosarcomas in mice and hamsters and liposarcomas and fibrosarcomas in rats [49].

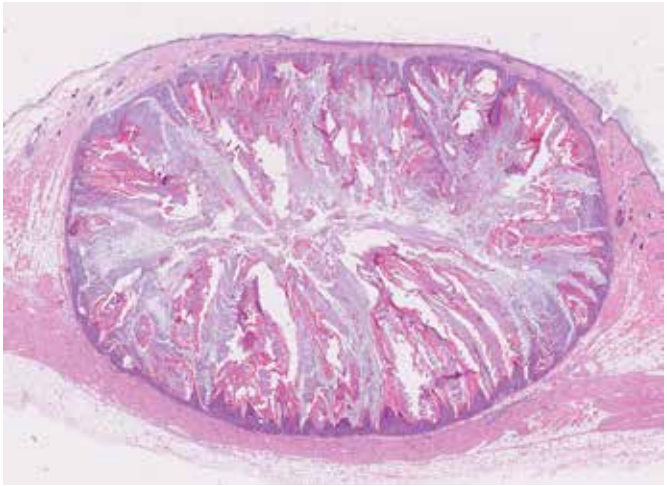

Figure 12. Animal model for human keratoacanthoma following a single intraperitoneal injection of *N*-methyl-*N*-nitrosourea in a male rat.

3.10. Other cutaneous toxicity due to systemic toxicity

3.10.1. Acne formation due to anti-inflammatory analgesics

This is a common clinical adverse reaction to NSAIDs or steroids and involves proliferation of acne bacteria leading to worsening of inflammation (steroid acne). In preclinical studies, spontaneous interdigit inflammation may worsen in beagle dogs following NSAID administration, eventually leading to skin ulcers in all extremities in severe cases (**Figure 13**).

Name of chemical compound	Route	Any skin carcinogenesis (2-year carcinogenesis study)				Mutagenicity (Ames)	TR No.
		F344 rats		B6C3F1 mice			
		Male	Female	Male	Female		
3-Amino-9-ethylcarbazole	Dietary	+	+	+	+	+	093
Benzene	Oral	CE	CE	CE	CE	–	289
Chloroethane	Inhalation	?	?	?	CE	+	346
C.I. acid red 114	Drinking water	CE	CE			+	405
C.I. basic red 9 monohydrochloride	Dietary	CE	CE	CE	CE	+	285
C.I. Direct Blue 15	Drinking water	CE	CE			–	397
2,4-Diaminoanisole sulfate	Dietary	+	+	+	+	+	084
3,3′-Dimethoxybenzidine dihydrochloride	Drinking water	CE	CE			+	372
3,3′-Dimethoxybenzidine-4,4′-diisocyanate	Dietary	+	+	–	–	+	128
3,3′-Dimethoxybenzidine dihydrochloride	Drinking water	CE	CE			+	390
2,4-Dinitrotoluene	Dietary	+	+	–	–	+	054
Fenthion	Dietary	–	–	?	–	+/–	103
Glycidol	Oral	CE	CE	CE	CE	+	374
Nithiazide	Dietary	–	+	+	?	+	146
5-Nitro-O-anisidine	Dietary	+	+	?	+	+	127
Nitrofurazone	Dietary	?	CE	–	CE	+	337
Rhodamine 6G	Dietary	?	?	–	–	–	364
Tris(aziridinyl)-phosphine sulfide	Subcutaneous	+	+	+	+	+	058
4-Vinyl-1-cyclohexene diepoxide	Dermal application	CE	CE	CE	CE	+	362

+: Positive, CE: apparent increase in incidence, ?: increased incidence but not significantly, –: negative, +/–: positive or negative, TR No.: National Toxicology Program (NTP) study number.
This table has been modified from [39].

Table 3. Chemical compounds reported to produce skin carcinogenesis from systemic exposure (US NTP study).

Figure 13. Nonsteroidal anti-inflammatory analgesic drug (NSAID)-induced skin lesion in a subacute toxicity study using beagle dogs. (a) Macroscopic observation of an interdigit lesion in the foot pad. Severe swelling and ulceration due to inflammation with local bacterial infection are observed. (b) In severe cases, the local lesions progress to skin ulcers on all extremities (subcutaneous phlegmon).

3.10.2. Drug-induced skin atrophy

Skin atrophy can be observed with long-term, repeated use of corticosteroids due to inhibitory effects on cell proliferation and/or fiber production, leading to decreases throughout the epidermis, skin appendages (hair follicles, sweat glands, and sebaceous glands) and subcutaneous adipose tissue [50] (**Figure 14**). Skin atrophy is also commonly observed with systemic exposure to anticancer drugs in preclinical studies.

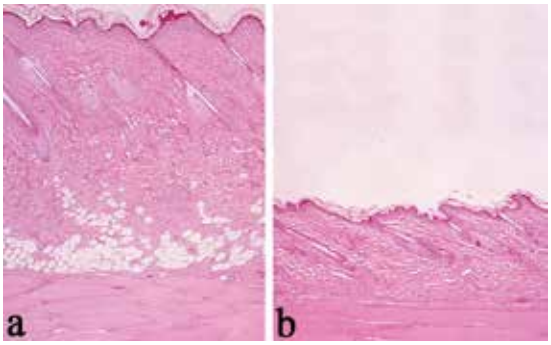

Figure 14. Corticosteroid-induced cutaneous atrophy in the rat. (a) In normal rat skin, the skin is thick with large hair shafts, sebaceous glands, and subcutaneous adipose tissue. (b) In the drug-exposed skin, the skin is thin with a severe decrease in all cutaneous layers, skin appendages (hair follicles and sebaceous glands), and subcutaneous adipose tissue.

3.10.3. Skin ulcer due to peripheral circulatory insufficiency

A lesion similar to cutaneous gangrene seen in diabetic patients can be induced in monkeys with certain drugs, and is speculated as a consequence of peripheral circulatory insufficiency due to the involvement of a vascular disorder [5].

3.10.4. Drug-induced granulomatous reaction

Hypodermic injections of certain drugs induce granulomatous inflammation located at the injection site, which is highly painful for the patients (**Figure 15**). Granulomas induced by luteinizing hormone-releasing hormone analogues have been reported in some patients for the treatment of prostatic cancers [51]. Histopathologically, epithelioid granulomatous inflammation with small vacuoles derived from the constituent ingredients of drug microcapsules has been observed [52]. In some patient cases, vaccinations induce granulomatous reactions at the injection site due to specific inflammation and irritation [53]. Recently, treatment with interferon has been associated with cutaneous granulomatous reactions and sarcoid reactions [54].

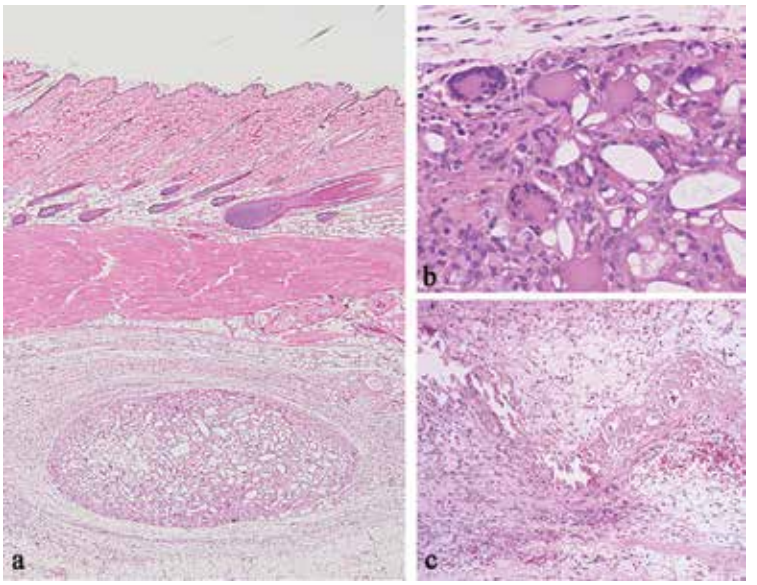

Figure 15. Drug-induced granuloma in subcutaneous tissue of the rat. (a) Granuloma is observed in the subcutaneous tissue. (b) Many foreign body giant cells that phagocytose lipoid materials are observed. Lipoid materials are derived from the contents of the drug. (c) Dystrophic calcification is observed in the obsolete lesions.

4. Closing remarks

This review has outlined the types and characteristics of drug-induced cutaneous toxicity, as well as providing descriptions of the methods of cutaneous toxicity testing required for safety

evaluation. It should be emphasized that cutaneous toxicity of drugs or chemical substances may appear in various forms. In recent years, advances have been made in the development of pharmaceutical products targeting specific molecules, genes, or nanotechnology-based pharmaceutical products. Due to the potential onset of cutaneous toxicity involving novel mechanisms with new pharmaceutical products, it will continue to be important to understand the basic toxic changes described here.

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References

[1] Mecklenburg L, Romeike A. Recommended diagnostic approach to documenting and reporting skin findings of nonhuman primates from regulatory toxicity studies. Toxicol Pathol 2016;44:591-600. DOI: 10.1177/0192623316638445.

[2] Sundberg JP, Nanney LB, Fleckman P, King LE. Skin and adnexa. In: Treuting PM Dintzis SM, editors. Comparative anatomy and histology A mouse and human atlas. London: Academic Press; 2012. p. 433–455.

[3] Li M, Urmacher CD. Normal skin. In: Mills SE, editor. Histopathology for pathologists, 3rd edition. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 3–28.

[4] Wojcinski ZW, Andrews-Jones L, Aibo DI, Dunstan R. Skin. In: Sahota PS, Popp JA, Hardisty JF, Gopinath C, editors. Toxicologic Pathology. Nonclinical Safety Assessment. New York: CRC Press; 2013. p. 831–893.

[5] Yoshizawa K. Drug-induced cutaneous toxicity. Folia Pharmacol Jpn 2008;131:285–290 (in Japanese). DOI: 10.1254/fpj.131.285.

[6] Yoshizawa K. Organ toxicity (respiratory toxicity, immunotoxicity, mucocutaneous toxicity, hematotoxicity and hematopoietic toxicity). The Japanese Society of Toxicology Workshop Materials in Fiscal 2010. The Japanese Society of Toxicology, 2010 (in Japanese).

[7] Rudmann D, Cardiff R, Chouinard L, Goodman D, Kuttler K, Marxfield H, Molonolo A, Treumann S, Yoshizawa K. Proliferative and nonproliferative lesions of the rat and mouse mammary, Zymbal's, preputial, and clitoral glands. Toxicol Pathol 2012;40(6 Suppl):7S-39S. DOI: 10.1177/0192623312454242.

[8] Yoshizawa K. Specialized sebaceous glands – Zymbal's gland, preputial gland, clitoral gland, and perianal gland. In: Suttie AW, Leininger JR, Bradley AE, editors. Boorman's Pathology of the Rats. Reference and Atlas. 2nd edition. London: Academic Press, 2016 (in press).

[9] Rice RH Cohen DE. Toxic response of the skin. In: Klaassen CD, editor. Casarett and Doull's Toxicology, 5th edition. New York: McGraw-Hill; 1996. p. 529–546.

[10] Burkhart C, Morrell D, Goldsmith L. Dermatological pharmacology. In: Goodman & Gillman's the Pharmacological Basis of Therapeutics, 12th edition (Japanese translation by Takaori S, Hashimoto K, Akaike A, Ishi K). Tokyo: Hirokawa Shoten; 2013. p. 2342–2378 (in Japanese).

[11] Mecklenburg L, Kusewitt D, Kolly C, Treumann S, Adans T, Diegel K, Yamate J, Kaufmann W, Muller S, Danilenko D, Bradley A. Proliferative and non-proliferative lesions of the rat and mouse integument. J Toxicol Pathol 2013;26(3 Suppl):27S–57S. DOI: 10.1293/tox.26.27S.

[12] Emoto Y, Yoshizawa K, Shikata N, Tsubura A, Nagasaki Y. Autopsy report for chemical burns from cresol solution. Exp Toxicol Pathol 2016;68:99–102. DOI: 10.1016/j.etp.2015.09.005.

[13] Emoto Y, Yoshizawa K, Shikata N, Tsubura A, Nagasaki Y. Autopsy results of a case of ingestion of sodium hydroxide solution. J Toxicol Pathol 2016;29:45–47. DOI: 10.1293/tox.2015-0049.

[14] Ng NY, Abdullah A, Milner SM. Cement burn. Eplasty 2015;15:ic13, eCollection 2015.

[15] Yoshizawa K, Heatherly A, Malarkey DE, Walker NJ, Nuska A. A critical comparison of murine pathology and epidemiological data of TCDD, PCB126, and PeCDF. Toxicol Pathol 2007;35:865–879. DOI: 10.1080/01926230701618516.

[16] Sorg O, Zennegg M, Schmid P, Fedosyuk R, Valikhnovskyi R, Gaide O, Kniazevych V, Saurat JH. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) poisoning in Victor Yushchenko: identification and measurement of TCDD metabolites. Lancet 2009;374:1179–1185. DOI: 10.1016/S0140-6736(09)60912-0.

[17] Nishigori C, Aoyama Y, Ito A, Suzuki K, Suzuki T, Tanemura A, Ito M, Katayama I, Oiso N, Kagohashi Y, Sugiura S, Fukai K, Funasaka Y, Yamashita

T, Matsunaga K. Guide for medical professionals (i.e. dermatologists) for the management of rhododenol-induced leukoderma. J Dermatol 2015;42:112–128. DOI: 10.1111/1346-8138.12744.

[18] Sasaki M, Kondo M, Sato K, Umeda M, Kawabata K, Takahashi Y, Suzuki T, Matsunaga K, Inoue S. Rhododendrol, a depigmentation-inducing phenolic compound, exerts melanocyte cytotoxicity via a tyrosinase-dependent mechanism. Pigment Cell Melanoma Res 2014;27:754–763. DOI: 10.1111/pcmr.12269.

[19] Tokuma Y, Fujiyama T, Ikeya S, Tatsuno K, Aoshima M, Kasuya A, Ito T. Biomedical, cytological, and immunological mechanisms of rhododendrol-induced leukoderma. J Dermatol 2015;77:146–149. DOI: 10.1016/j.jdermsci.2015.01.017.

[20] Pharmaceuticals and Medical Devices Agency (PMDA). Safety Information for Pharmaceutical Products and Medical Devices No. 261, 2009. Available from: https://www.pmda.go.jp/files/000153275.pdf [accessed: 2016-04-30].

[21] Macdonald JB, Macdonald B, Golotz LE, LoRusso P, Sekulic A. Cutaneous adverse effects of targeted therapies. Part I. Inhibitors of the cellular membrane. J Am Acad Dermacol 2015;72:203–218. DOI: 10.1016/j.jaad.2014.07.032.

[22] Reyes-Habito CM, Roh EK. Cutaneous reactions to chemotherapeutic drugs and targeted therapies for cancer. Part II. Targeted therapies. J Am Acad Dermatol 2014;71:217 e1–11. DOI: 10.1016/j.jaad.2014.04.013.

[23] Maraschin R, Bussi R, Conz A, Orlando L, Pirovano R, Nyska A. Toxicological evaluation of u-hEGF. Toxicol Pathol 1995;23:356–366. DOI: 10.1177/019262339502300312.

[24] Gutzmer R, Wollenberg A, Ugurel S, Homey B, Ganser A, Kapp A. Cutaneous side effects of new antitumor drugs: clinical features and management. Dtsch Arztebl Int 2012;109:133–140. DOI: 10.3238/arztebl.2012.0133.

[25] BelliniV, Bianchi L, Pelliccia S, Lisi P. Histopathologic features of erythematous papulopustular eruption to epidermal growth factor receptor inhibitors in cancer patients. J Cutan Pathol 2016;43:211–218. DOI: 10.1111/cup.12630.

[26] Li T, Perez-Soler R. Skin toxicities associated with epidermal growth factor receptor inhibitors. Targ Oncol 2009;4:107–119. DOI: 10.1007/s11523-009-0114-0.

[27] Piraccini BM, Iorizzo M, Rech G, Tosti A. Drug-induced hair disorders. Curr Drug Saf 2006;1:301–305. DOI: 10.2174/157488606777934477.

[28] Welle MM, Wiener DJ. The hair follicle: a comparative review of canine hair follicle anatomy and physiology. Toxicol Pathol 2016;44:564-574. DOI: 10.1177/0192623316631843.

[29] Hendrix S, Handjiski B, Peters EM, Paus R. A guide to assessing damage response pathways of the hair follicle: lessons from cyclophosphamide-induced alopecia in mice. J Invest Dermatol 2005;125:42–51. DOI: 10.1111/j.0022-202X.2005.23787.x.

[30] Wikramanayake TC, Amini S, Simon J, Mauro LM, Elgart G, Schachner LA, Jimenez JJ. A novel rat model for chemotherapy-induced alopecia. Clin Exp Dermatol 2012;37:284–289. DOI: 10.1111/j.1365-2230.2011.04239.x.

[31] Yoshizawa K, Nambu H, Yamamoto D, Yang J, Kiyozuka Y, Shikata N, Tsubura A. Time-specific occurrence of alopecia in neonatal C57BL mice treated with *N*-methyl-*N*-nitrosourea and the therapeutic efficacy of tacrolimus hydrate. Pathol Int 2000;50:175–184. DOI: 10.1046/j.1440-1827.2000.01021.x.

[32] Ponticelli C, Bencini PL. Nonneoplastic mucocutaneous lesions in organ transplant recipients. Transpl Int 2011;24:1041–1050. DOI: 10.1111/j.1432-2277.2011.01308.x.

[33] Rhodes L, Harper J, Uno H, Gaito G, Audette-Arruda J, Kurata S, Berman C, Primka R, Pikounis B. The effects of finasteride (Proscar) on hair growth, hair cycle stage, and serum testosterone and dihydrotestosterone in adult male and female stumptail macaques (Macaca artoides). J Clin Endocrinol Metab 1994;79:991–996. DOI: 10.1210/jcem.79.4.7962310.

[34] Piraccini B, Starace M, Alessandrini A, Guarrera M, Fiorucci MC, Lorenzi S. Efficacy and tolerability of 5% minoxidil solution (Carexidil[R]) in male and female androgenetic alopecia: a 6-month open multicentric study. G Ital Dermatol Venereol 2011;146:1–8 (article in Italian).

[35] Alm A. Latanoprost in the treatment of glaucoma. Clin Opthalmol 2014;8:1967–1985. DOI: 10.2147/OPTH.S59162.

[36] Gilbar P, Hain A, Peereboom VM. Nail toxicity induced by cancer chemotherapy. J Oncol Pharm Pract 2009;15:143–155. DOI: 10.1177/1078155208100450.

[37] Archier E, Devaux S, Castela E, Gallini A, Aubin F, Le Maitre M, Aractingi S, Bachelez H, Cribier B, Joly P, Jullien D, Misery L, Paul C, Ortonne JP, Richard MA. Carcinogenic risks of psoralen UV-A therapy and narrowband UV-B therapy in chronic plaques psoriasis: a systemic literature review. J Eur Acad Dermatol Venereol 2012;26(Suppl 3): 22–31. DOI: 10.1111/j.1468-3083.2012.04520.

[38] Stern RS, Nichols KT, Vakeva LH. Malignant melanoma in patients treated for psoriasis with methoxsalen (psoralen) and ultraviolet A radiation (PUVA). The PUVA follow-up study. N Engl J Med 1997;336:1041–1045. DOI: 10.1056/NEJM199704103361501.

[39] Huff J, Cirvello J, Haseman J, Bucher J. Chemicals associated with site-specific neoplasia in 1394 long-term carcinogenesis experiments in laboratory rodents. Environ Health Perspect 1991;93:247–270.

[40] Mitamura T, Doi Y, Kawabe M, Lilja H, Motomura M, Oishi Y, Yoshizawa K, Seki J. Inhibitory potency of tacrolimus ointment on skin tumor induction in a mouse model of an initiation-promotion skin tumor. J Dermatol 2011;38:562–570. DOI: 10.1111/j.1346-8138.2010.01046.x.

[41] Lynch D, Svoboda J, Putta S, Hofland HE, Chern WH, Hansen LA. Mouse skin models for carcinogenic hazard identification: utilities and challenges. Toxicol Pathol 2007;35:853–864. DOI: 10.1080/01926230701748131.

[42] Aldaz CM, Conti CJ, Klein-Szanto AJ, Slaga TJ. Progressive dysplasia and aneuploidy are hallmarks of mouse skin papilloma: relevance to malignancy. Proc Natl Acad Sci U S A. 1987;84:2029–2032.

[43] Satoh M. Analysis of toxicity using metallothionein knockout mice. Yakugaku Zasshi 2007;127:709–717 (abstract in English, text in Japanese). DOI: 10.1248/yakushi.127.709.

[44] Zilfou JT, Lowe SW. Tumor suppressive functions of p53. Cold Spring Harb Perspect Biol 2009;1:a0001883. DOI: 10.1101/cshperspect.a001883.

[45] Page A, Navarro M, Suarez-Cabrera C, Alameda JP, Casanova ML, Paramio JM, Bravo A, Ramirez A. Protective role of p53 in skin cancer: carcinogenesis studies in mice lacking epidermal p53. Oncotarget 2016 (in press). DOI: 10.18632/oncotarget.7897.

[46] Tennant RW, Stasiewicz S, Eastin WC, Mennear JH, Spalding JW. The Tg.AC (v-Ha-ras) transgenic mouse: nature of the model. Toxicol Pathol 2001;29(Suppl):51–59. DOI: 10.1080/019262301753178474.

[47] Yuki M, Yoshizawa K, Emoto Y, Yuri T, Kinoshita Y, Tsubura A, Kurokawa I. Cutaneous epithelial lesions induced by *N*-methyl-*N*-nitrosourea in male Sprague-Dawley rats: a possible animal model for human keratoacanthoma. Anticancer Res 2016;36:111–120.

[48] Ramselaar CG, Ruitenberg EJ, Kruizinga W. Regression of induced keratoacanthomas in anagen (hair growth phase) skin grafts in mice. Cancer Res 1980;40:1668–1673.

[49] Hardisty JF, Elwell MR, Ernst H, Greaves P, Kolenda-Roberts H, Malarkey DE, Mann PC, Tellier PA. Histopathology of hemangiosarcoma in mice and hamsters and liposarcoma/fibrosarcomas in rats in rats associated with PPAR agonists. Toxicol Pathol 2007;35:928–941. DOI: 10.1080/01926230701748156.

[50] Hisatomi A, Mitamura T, Kimura M, Oishi Y, Fujii T, Ohara K. Comparision of FK506 (Tacrolimus) and glucocorticoid ointment on dermal atrophogenicity in rats. J Toxicol Pathol 1997;10:97–102. DOI: 10.1293/tox.10.97.

[51] Shiota M, Tokuda N, Kanou T, Yamasaki H. Incidence rate of injection-site granulomas resulting from the administration of luteinizing hormone-releasing hormone analogues for the treatment of prostatic cancer. Yonsei Med J 2007;48:421–424. DOI: 10.3349/ymj.2007.48.3.421.

[52] Watanabe T, Yamada N, Yoshida Y, Yamamoto O. Granulomas induced by subcutaneous injection of a luteinizing hormone-releasing hormone analog: a case report and review of the literature. J Curan Pathol 2010;37:1116–1118. DOI: 10.1111/j.1600-0560.2009.01456.x.

[53] Rosenblatt AE, Stein SL. Cutaneous reactions to vaccinations. Clin Dermatol 2015;33:327–332. DOI: 10.1016/j.clindermatol.2014.12.009.

[54] El-Khalawany M, Mohammad I, Aboeldahab S, Thabet A. Cutaneous granulomas associated with interferon therapy. Am J Dermatopathol 2016 (in press). DOI: 10.1097/DAD.0000000000000547.

Chapter 2

Hepatic and Intestinal Multidrug Resistance-Associated Protein 2: Transcriptional and Post-transcriptional Regulation by Xenobiotics

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Additional information is available at the end of the chapter

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Abstract

We are daily exposed to a large number of pharmacological drugs, environmental pollutants, and natural toxins, which represent a potential toxic insult. The organism possesses a sophisticated system of detoxification particularly expressed in the liver, intestine, and kidney. This system consists of intracellular biotransformation enzymes that convert the toxins into more hydrophilic derivatives followed by their elimination through membrane transporters. Multidrug resistance-associated protein 2 (MRP2, ABCC2) is an important member of the ATP-binding cassette (ABC) superfamily of transporters localized at the apical membrane of polarized cells, such as hepatocytes, enterocytes, and renal tubular cells. MRP2 is proposed as a major actor in the elimination of endo- and xenobiotics, mainly conjugated with glucuronic acid, glutathione, and sulfate. The small intestine and the liver constitute relevant detoxification organs expressing MRP2 and therefore preventing absorption and promoting the hepatobiliary clearance of xenobiotics. MRP2 expression and/or function can be modulated by therapeutic drugs, herbal products, dietary compounds, and environmental pollutants. Consequently, MRP2 modulation could cause changes in tissue exposure, with potential toxicological and pharmacological consequences. This chapter reviews the information available on the role of hepatic and intestinal MRP2 in detoxification processes, and their regulation by xenobiotics, considering in particular its toxicological relevance.

Keywords: MRP2, intestine, liver, detoxification, clearance, toxicity

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1. Introduction

1.1. Detoxification of xenobiotics

Most organisms are daily exposed to a large number of different xenobiotics, such as therapeutic drugs, environmental pollutants, and natural toxins. The major routes of exposure to these chemicals comprise incorporation with the diet, inhalation or absorption through the skin. Preservation of health depends largely on the body's ability to eliminate these harmful substances. In this regard, the organism possesses a sophisticated system of detoxification mainly expressed in organs such as the liver, intestine, and kidney. The detoxification process consists of intracellular biotransformation enzymes that neutralize the toxins and membrane transporters for their subsequent elimination from the cell. The biotransformation process is carried out by the same biochemical machinery that metabolizes endogenous compounds, often of similar chemical structure. Even though a compound can be excreted without undergoing any change, it is usually converted by biotransformation enzymes into a more hydrophilic derivative prior to elimination. The biotransformation reactions are carried out by phase I enzymes such as cytochrome P450 (CYP) members and by phase II conjugating enzymes such as glutathione S-transferase (GST; EC 2.5.1.18), UDP-glucuronosyltransferase (UGT; EC 2.4.1.17), and sulfotransferase (SULT; EC 2.8.2.) [1, 2]. Phase I enzymes usually act in tandem with phase II enzymes, which ultimately results in incorporation of anionic groups into the xenobiotic molecule. The hydrophilic derivatives can be excreted from the cells by phase III or membrane transport systems, with the anionic groups acting as *affinity markers* for a series of transporters of the multidrug resistance-associated protein (MRP) family. These proteins are members of the ATP-binding cassette (ABC) drug efflux transporters and mediate the active extrusion of biotransformation products for their subsequent elimination from the body [1, 2]. Direct elimination of xenobiotics without suffering any biotransformation is also possible and is denominated *"phase 0 metabolism"* [3].

What follows is a description of the role of the multidrug resistance-associated protein 2 (MRP2, ABCC2) as an important component of the ABC family involved in xenobiotic disposition by liver and intestine.

1.2. Multidrug resistance-associated protein 2 and its role in the detoxification of xenobiotics

MRP2 structure consists of a large core segment, containing two cytosolic nucleotide-binding domains (NBDs), two membrane-spanning domains (MSDs), and a linker segment L1, shared by other ABC members. Additionally, MRP2 contains a third NH_2-terminal membrane-spanning domain MSD0, also called terminal transmembrane domain, with five transmembrane helices resulting in an extracellular NH_2-terminus and an intracellular linker segment 0 (L0) (**Figure 1**) [4]. MRP2 is characteristically expressed at the apical membrane of polarized cells such as hepatocytes, enterocytes, and renal tubular cells [4–6], where it plays a primary role in the elimination of specific compounds. Particularly, the highest concentration of this transporter is found in the liver and intestine [7], two organs playing a prominent role in xenobiotic detoxification commonly known as "first-pass metabolism and clearance". Protein expression of MRP2 is highest in enterocytes of the proximal small intestine, decreasing in

direction to the terminal ileum [8], gradient shared with the expression of phase II metabolizing enzymes such as GST and UGT [9, 10], thus suggesting that metabolism and transport processes may act coordinately. In the liver, the major biotransformation organ, MRP2 is abundantly expressed in the bile canalicular membranes of the hepatocyte [11], where it plays a role in bile formation through secretion of endogenous substrates such as glutathione (GSH) and glutathione conjugates. Canalicular MRP2 also constitutes the main route of elimination of xenobiotics conjugated with GSH, sulfate or glucuronic acid [12]. Similarly, immunohistochemical analysis in normal rat liver demonstrated that UGTs and rMrp2 are localized in the same regions [13, 14], also indicating that they may work in cooperation. MRP2 in either localization contributes significantly to disposition of potentially harmful compounds thus decreasing their toxicity.

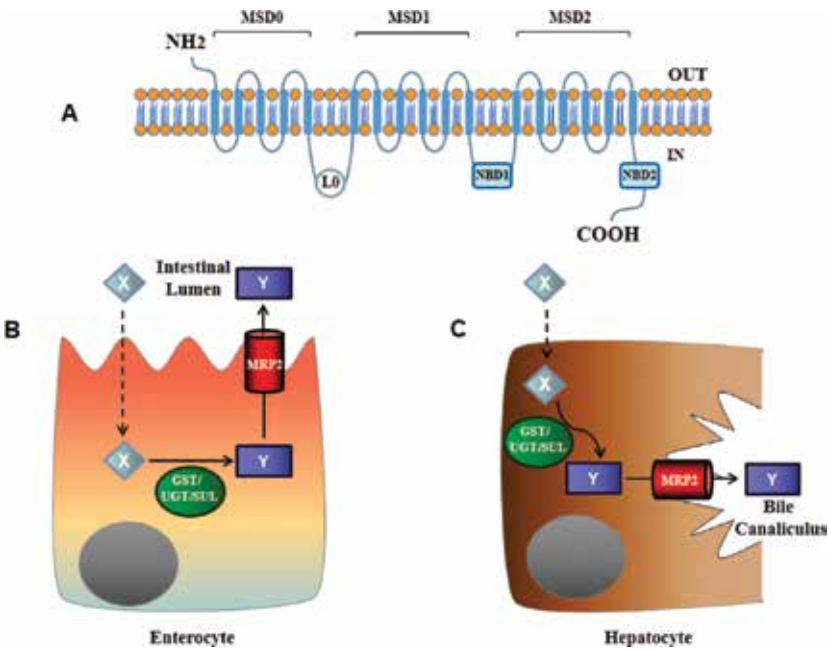

Figure 1. (A) Molecular structure of MRP2/ABCC2. MSDs: membrane-spanning domains; NBDs: nucleotide-binding domains; L0: linker segment 0. (B) Coordinated action between phase II metabolizing enzymes and MRP2 in the enterocyte. Hydrophobic xenobiotics (X) may enter the cell by diffusion through the apical membrane of the enterocyte. After that, they may suffer metabolic phase I reaction by cytochrome P450 and/or subsequent conjugation by phase II enzymes, such as UDP-glucuronosyltransferase (UGT) localized in the endoplasmic reticulum or glutathione S-transferase (GST) or sulfotransferase (SUL) localized in the cytosol. In the phase III, the more hydrophilic metabolites (Y) may be actively secreted into the intestinal lumen by MRP2. (C) Coordinated action between phase II metabolizing enzymes and MRP2 in the hepatocyte. Here, hydrophobic xenobiotics (X) enter the cell through the basolateral pole of the membrane in the hepatocyte. After conjugation by phase II metabolizing enzymes, the final product (**Y**) may be secreted into bile by MRP2.

Selected substrates of MRP2 are included in **Table 1**. Studies using Groningen Yellow (GY)/ TR- Wistar [15, 16] or Eisai hyperbilirubinemic rats (EHBR) [17] that are rMrp2 deficient as a result of mutations leading to premature stop codons have helped to identify transporter

substrates initially. Interestingly, ingredients of our daily diet are substrates for MRP2. That is the case of the tea component epicatechin, the dietary supplement chrysin [18, 19], and the meat-derived dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) [20]. Finally, it is important to emphasize that there are MRP2 substrates which influence transporter expression thus influencing its own bioavailability. That is the case, for example, of tamoxifen [21].

Endogenous compounds	Exogenous compounds
Glutathione	*Anticancer drugs*: doxorubicin, epirubicin, etoposide, irinotecan, methotrexate,
Bilirubin glucuronides	mitoxantrone, cisplatin, tamoxifen, vincristine, vinblastine, camptothecin
Conjugated bile salts	*Antibiotics*: ampicillin, azithromycin, cefodizime, ceftriaxone, grepafloxacine,
Leukotrienes C4, D4, E4	irinotecan
Steroids	*HIV drugs*: adenovir, cidofovir, indinavir, lopinavir, nelfinavir, ritonavir,
(17β-glucuronosyl estradiol)	saquinavir
Triiodo-L-thyronine	*Other drugs*:genistein-7-glucoside, p-aminohippurate, olmesartan, phloridzin,
ethinylestradiol-3-O-glucuronide	pravastatine, quercetin 4′-β-glucoside, temocaprilate, conjugates of
	acetaminophen, indomethacin, phenobarbital, sulfinpyrazone.
	Toxicants: S-glutathionyl-2,4-dinitrobenzene, S-glutathionyl ethacrynic acid,
	ochratoxin A, 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridin, 4-
	(methylnitrosamino)-1-(3-pyridyl)-1-buta-nol, α-naphtylisothio-cyanate,
	heavy metal complexes (arsenic glutathione, Sb, Zn, Cu, Mn, Cd)
	Dyes: fluo-3, carboxydichloro fluorescein, sulfobromophthalein
	Flavonoids: epicatechin, chrysin

Table 1. Selected MRP2 substrates.

2. Regulation of expression and activity of MRP2 by xenobiotics

Expression of MRP2 can be regulated at different levels [22], which can be grouped in two main categories: (i) transcriptional level, implying changes in mRNA synthesis rate, or (ii) post-transcriptional level, comprising complex processes involving or not changes in MRP2 mRNA levels. Regarding the transcriptional regulation, the MRP2 promoter contains a number of binding sites recognizing a variety of transcription factors, which in turn can be activated by either endo- or xenobiotics [23, 24]. Thus, a wide variety of drugs, environmental pollutants, and natural toxins behave as ligands of transcription factors/nuclear receptors such as farnesoid X-activated receptor (FXR), pregnane X receptor (PXR), liver X receptor α (LXRα), and constitutive androstane receptor (CAR), to ultimately induce the synthesis of MRP2 mRNA [25]. They modulate MRP2 transcriptionally through binding to the response element

ER-8, which extends from −401 to −376 bp of the MRP2 promoter [25]. Additionally, the study of MRP2 promoter identified binding sites for other transcription factors such as the nuclear factor-erythroid 2-related factor-2 (Nrf2), the peroxisome proliferator-activated receptor alpha (PPARα), CCAAT/enhancer-binding protein-β (C/EBPβ), and hepatic nuclear factors (HNFs), which can also influence the MRP2 expression at the transcriptional level. Finally, a particular transcriptional regulation involves the intracellular nucleotide cyclic adenosine monophosphate (cAMP) pathway, likely triggered by xenobiotics acting predominantly through binding to plasma membrane receptors. This was so far demonstrated in the intestine [26], where the treatment of Caco-2 human intestinal cells with the membrane-permeable analogue dibutyryl cAMP or the adenylyl cyclase activator forskolin led to a significant induction in hMRP2 protein and mRNA expression. Reporter gene and chromatin immunoprecipitation assays performed in this same study showed an increased binding of the transcription factors c-JUN and activating transcription factor-2 (ATF2) to a regulatory region containing activator protein-1 (AP-1) and cAMP response element (CRE) binding sites within the MRP2 promoter.

On the other hand, post-transcriptional MRP2 regulation can involve the dynamic endocytic retrieval and exocytic insertion of this transporter between the canalicular membrane and an intracellular pool of vesicles [27]. A variety of signal transduction pathways involving the activation of the mitogen-activated protein kinases (MAPK) A and C take part in these events [27–29]. Also, mRNA splicing may account for post-transcriptional regulation. As an example, alternative splicing of hMRP2 mRNA has been shown to be a cause underlying Dubin–Johnson syndrome as a consequence of synthesis of nonfunctional protein [30, 31]. Additionally, MRP2 can be translationally modulated. In this regard, Jones et al. [32] observed that under certain situations rMrp2 protein in rats is modified without changes in mRNA levels. This is not just attributed to a modified rate of protein degradation but to the presence of several rMrp2 transcription initiation sites in the 5' untranslated region [7, 33]. The alternative use of these sites leads to the production of different rMrp2 mRNAs with differential translational efficiency.

What follows is a description of the regulatory properties of MRP2 as an important component of the ABC family in liver and intestine. The effects of xenobiotics on MRP2 expression and activity were particularly considered. Transcriptional and post-transcriptional regulations were described separately.

2.1. Transcriptional regulation

Several xenobiotics including therapeutic drugs, environmental pollutants, and natural toxins have shown to activate different transcription factors and nuclear receptors thus exhibiting the potential of increasing MRP2 expression [34]. However, not all xenobiotics induce the expression of MRP2. Indomethacin, a nonsteroidal anti-inflammatory drug, reduced the expression of rat rMrp2 at the mRNA and protein levels in the liver [35]. This reduction was associated with a diminished mRNA expression of the hepatic nuclear receptors CAR, FXR, PXR, retinoic acid receptor α (RARα), and retinoid X receptor α (RXRα). This down-regulation of nuclear receptors is consistent with observations in endotoxin-treated rats that also proved to cause rMrp2 down-regulation at the transcriptional level [36, 37]. Intestinal injury caused

by indomethacin can increase endotoxin levels in portal blood [38], which in turn induces several immune responses and oxidative stress, as shown by the reduced levels of hepatic GSH and increased levels of nitric oxide (NO) and nitrosothiols in portal blood [35]. In this regard, El Kasmi et al. demonstrated that dextran sulfate sodium-induced intestinal injury also down-regulates hepatic mMrp2 expression in mice liver and that the intestinal microbiota and TLR4 (Toll-like receptor 4) are involved in this effect [39]. In addition, it was shown that mice with intestinal injury that received soy oil-based parenteral nutrition containing phytosterols exhibited an exacerbated decrease in mMrp2 mRNA levels. The phytosterol stigmasterol was at least partially involved and associated with increased levels of interleukin 6 (IL-6) mRNA and reduced levels of FXR mRNA in liver [39].

In contrast to indomethacin, several drugs are able to induce the expression of MRP2. For example, spironolactone (SL), a drug used to treat patients with edema and ascites, has been shown to increase bile flow in rats due to the up-regulation of rMrp2 at the transcriptional level, probably in response to increased PXR levels [40]. This up-regulation in rMrp2 resulted in increased efflux activity of the model substrate of rMrp2 dinitrophenyl-S-glutathione (DNP-SG) *in vitro*. These findings were in accordance with observations in patients exhibiting increased clearance of drugs co-administered with SL [41, 42]. Benznidazole (BZL), a nitroimidazole administered for treatment of Chagas disease, was also shown to induce rMrp2 protein expression [43] in rats. This induction is presumably mediated by PXR, since knock-down of the nuclear receptor in the hepatic cell line HepG2 prevented the induction of hMRP2 by this drug [44]. The antituberculosis agents rifampicin and isoniazid that cause liver injury also up-regulate hepatic rMrp2 protein expression in rats [45]. Interestingly, monoammonium glycyrrhizin, commonly present in Chinese herbal formulas used for hepatic protection, prevented such increases in rMrp2 expression. In addition rifampicin and isoniazid increased lipid peroxidation and GSH levels in hepatic tissue, indicating the presence of oxidative stress, which was also prevented by monoammonium glycyrrhizin.

Acetaminophen (APAP) represents one of the most common over-the-counter drugs, used as an effective and safe analgesic and antipyretic. Nevertheless, APAP overdose is very frequent and associated with severe liver injury. Although the mechanism underlying APAP toxicity is not completely understood, the CYP-biotransformation product N-acetyl-p-benzoquinone imine (NAPQI) was described as a mediator of APAP toxic effects since it promotes GSH depletion and binds itself to cellular proteins [46]. APAP phase II metabolites resulting from conjugation with glucuronic acid and sulfate are known MRP2 substrates. Similarly, MRP2 transports the GSH conjugate of NAPQI, thus contributing to reduce the toxic burden exerted by APAP. In this regard, the administration of a single hepatotoxic dose of APAP to Wistar rats resulted in an increase in rMrp2 expression in liver plasma membranes [47]. In a similar study, an induction hepatic mMrp2 following Nrf2 activation was demonstrated in mice, clearly suggesting the presence of an adaptive mechanism to the APAP-triggered injury [48, 49]. In line with these observations, therapeutic activation of Nrf2 has been proposed as a possible strategy to ameliorate APAP-associated hepatotoxicity [50–52]. Nrf2 bears a special toxicological relevance due to its activation by pro-oxidant compounds and reactive metabolites usually associated with situations of drug overdose or exposure to environmental toxicants. Under

homeostatic conditions, Nrf2 is sequestered in the cytosol by the Kelch-like ECH-associated protein 1 (Keap1) which promotes Nrf2 ubiquitination and proteosomal degradation. Upon an oxidative stimulus, it takes place a modification in the oxidation status of particular cysteine residues in the Keap1 molecule leading to Nrf2 dissociation and migration to the nucleus where it binds to antioxidant response elements (ARE) within the promoters and activates the transcription of target genes [53] including antioxidant and GSH synthesis enzymes and also drug transporters like MRP2 [54]. Management of Nrf2 activation could be applied to other cases of oxidative stress-associated hepatic injury. For instance, the Nrf2 activator N-acetyl-cysteine was described to ameliorate the rMrp2 down-regulation exerted by the pro-oxidant phytochemical timosaponin A3 [55]. Similarly, other Nrf2 activators are being tested in clinical trials for the treatment of hepatic and extrahepatic diseases [53].

Environmental pollutants such as arsenite significantly increased rMrp2 protein expression in rat liver after 2 weeks of exposure. Longer exposure treatment (4 or 6 weeks) also increased rMrp2 expression but to a lesser extent [56]. Arsenite not only regulates Mrp2 but is also an MRP2 substrate, so transporter induction may help to counteract the toxic effects of arsenite in the liver. In agreement with this, arsenite content in bile decreased with the exposure time in the same manner as rMrp2 protein induction. Lipid peroxidation was increased and GSH peroxidase activity was reduced in the liver at 4 and 6 weeks of arsenite exposure, indicating a probable effect of oxidative stress in attenuating hepatic rMrp2 induction. This regulation of Mrp2 may explain the dual effects reported for arsenite exposure [57].

The T-2 mycotoxin is commonly found in different crops. Prolonged exposure (3 weeks) of poultry to T-2 reduced cMrp2 mRNA expression in the liver of broiler chickens [58]. Although the authors suggest that PXR may be involved in the down-regulation observed, no studies were conducted to prove that. The organochlorine pesticides 2,4'-dichlorodiphenyltrichloroethane (DDT), 4,4'-DDT, chlordane, heptachlor, dieldrin, and lindane, highly resistant to degradation, were shown to increase hMRP2 mRNA in HepaRG cells after a 48-h exposure [59]. This regulation could be mediated by PXR, since this nuclear receptor expression was increased in human hepatocytes after treatment with chlordane, dieldrin, and endosulfan [60].

Bisphenol A (BPA) is a typical contaminant of food, water, and air. A study evaluating the association between the expression of drug transporters in fetal liver and BPA exposure showed a positive correlation between BPA levels and hMRP2 expression. A similar correlation was described between BPA levels and Nrf2 expression, suggesting also a mediation of this transcription factor in the hMRP2 regulation by BPA and agreeing well with a previous report showing Nrf2 activation by BPA *in vitro* [61, 62].

Transcriptional regulation of MRP2 by xenobiotics was also demonstrated in extrahepatic tissues like the small intestine. An example of therapeutic drugs affecting MRP2 gene expression is rifampicin, a well-known PXR agonist which has been demonstrated to increase hMRP2 expression and activity in healthy volunteers. Fromm et al. reported an up-regulation of hMRP2 mRNA and protein in duodenal biopsies [63]. Later, Oswald et al. showed the impact of this regulation on oral availability and efficacy of the coadministered drug ezetimibe [64]. The same inducing effect was reported using the human-derived cell line LS180 [65]. Similarly, carbamazepine is another PXR agonist [66] shown to increase hMRP2 mRNA and protein levels

in human healthy volunteers and also to reduce intestinal absorption of the co-administered MRP2 substrate talinolol [67].

Even though there are only few reports on drug–drug interactions associated with PXR-dependent modulation of intestinal MRP2, many other agents have been shown to regulate its expression via PXR. Pregnenolone-16α-carbonitrile, a synthetic steroid, induced mMrp2 mRNA expression in jejunum of C57BL/6 mice (200 mg/kg, i.p., 4 days). A concomitant up-regulation of PXR mRNA was observed and the involvement of this nuclear receptor was confirmed using PXR knockout mice [68]. The antiviral agents and PXR agonists efavirenz and saquinavir [69] also up-regulated hMRP2 expression in LS180 cells [65, 70]. Since these drugs are usually involved in long-term treatments, coadministration with MRP2 substrates could result in unwanted drug–drug interactions. In the same human intestinal cell line, the effect of the endothelin receptor antagonist bosentan [71] and the antineoplastic mitotane [72] was demonstrated. They both exerted a significant hMRP2 induction concomitantly with PXR activation at pharmacologically relevant concentrations.

In some studies, the increases in MRP2 expression were correlated experimentally with increased transport activity. Examples of these drugs are BZL and SL, both PXR agonists. Perdomo et al. reported an induction of rMrp2 protein in jejunum of BZL-treated rats (100 mg/kg, i.p., 3 days) [43]. This change was accompanied with an increased efflux of DNP-SG to the intestinal lumen. These results not only demonstrate higher secretion of rMrp2 substrates but also strongly suggest a restriction in the absorption of xenobiotics incorporated luminally in BZL-treated rats. Similarly, SL was demonstrated to increase serosal to mucosal transport of DNP-SG well correlating with increased rMrp2 mRNA and protein expression in rat proximal jejunum. The PXR antagonist ketoconazole was able to prevent this induction, suggesting mediation by this nuclear receptor [73].

Cimetidine and quinidine have been shown to increase both hMRP2 and PXR expressions in T84 cells [74]. Similarly, the anticonvulsant phenobarbital induced PXR mRNA and hMRP2 mRNA and protein at the same time in Caco-2 cells [75]. On the contrary, MRP2 expression is down-regulated by drugs that decrease PXR expression. Haslam et al. reported such an effect in T84 cells for the cholesterol-lowering drug atorvastatin and the anticancer agents topotecan and irinotecan [74].

Xenobiotics can regulate expression of intestinal MRP2 interacting with nuclear receptors other than PXR. That is the case of the bcl-2 inhibitor obatoclax, which at nanomolar concentrations induced hMRP2 mRNA in LS180 cells through activation of the aryl hydrocarbon receptor [76]. Another example is the proteasome inhibitor bortezomib, which also increases hMRP2 mRNA expression in SW-480 human cells [77]. Even though the mediator has still to be identified, the transcription factor Nrf2 appears to be the main candidate considering its simultaneous up-regulation and also the well-demonstrated relationship between MRP2 induction and Nrf2 activation in tissues like liver [49], kidney [78], and brain [79].

Among the natural compounds modulating intestinal MRP2, the isothiocyanates sulforaphane (SF) and erucin (ER) were well studied. Derived from cruciferous vegetables, they have drawn the attention of the researchers due to their anticancer properties [80]. After microarray studies,

Traka et al. reported hMRP2 up-regulation in Caco-2 cells treated with SF (50 μM, 24 h) [81]. They confirmed this finding by RT-PCR analysis. Using the same model, Jakubíková et al. showed a similar hMRP2 mRNA increase after treatment with SF and with ER (20 μM, 24 h) [82]. This effect appears to be partly mediated by phosphoinositide 3-kinase (PI3K)/AKT, considering the inhibition exhibited by the PI3K/AKT inhibitor LY294002. Later, in analogous experimental conditions, Harris and Jeffery demonstrated an induction also at protein level [83]. Considering that Nrf2 is activated by SF [84], this transcription factor may play a role in MRP2 up-regulation.

Other naturally occurring xenobiotics found to modulate intestinal MRP2 are the polyphenols quercetin and resveratrol (RES). The first one was demonstrated to up-regulate hMRP2 at protein level in Caco-2 cells (50 and 100 μM, 72 h). A coordinated induction of the phase II enzyme UGT1A6 was also observed, suggesting reduced absorption and enhanced secretion of glucuronides at intestinal level [85]. Studies using RES have become of strong interest after the increasing evidence regarding its beneficial health effects. A short time ago, RES ability to down-regulate MRP2 at mRNA and protein levels has been proven in rat intestine and Caco-2 cells [86]. The functional impact was evaluated in the latter model by determination of intracellular retention of the MRP2 substrate methotrexate (MTX). Treatment with RES increased the accumulation of MTX, suggesting a suppressed efflux activity. Moreover, in an attempt to clarify the mechanism, the authors found a concomitant inhibition of the insulin-like growth factor receptor 1 (IGF-1R)/AKT/ERK signaling pathway.

2.2. Post-transcriptional regulation

As anticipated, post-transcriptional regulation of MRP2 activity may also occur in response to exposition to xenobiotics. Faldaprevir is a drug used to treat patients with hepatitis C in spite that it causes hyperbilirubinemia. Acute treatment of human and rat hepatocytes with faldaprevir inhibited hMRP2-/rMrp2-mediated efflux of bilirubin glucuronides into bile [87]. This may partially explain the impaired bilirubin disposition by faldaprevir. However, toxicology studies in monkeys and patients showed that the bilirubin accumulated during faldaprevir treatment was mainly unconjugated, suggesting that the main cause is probably inhibition of glucuronidation ather than excretion of the conjugated metabolites. Simeprevir, another drug used to treat hepatitis, causes hyperbilirubinemia composed by conjugated and unconjugated bilirubin, which supports an impaired hMRP2 efflux activity [88].

Ethynylestradiol (EE) and genistein (GNT) are estrogenic compounds of particular relevance considering their oral incorporation as components of contraceptives formulations and soy-derived food, respectively. Interestingly, they were demonstrated to regulate intestinal MRP2 at post-transcriptional level, although showing a noticeable dose and model dependence. Thus, it was initially found that EE at a cholestatic dose (5 mg/kg b.w. day, for 5 consecutive days, s.c.) down-regulates rMrp2 expression and function in rat proximal jejunum, without changes in mRNA levels [89]. However, at pharmacological concentrations (0.5–5 pM) EE up-regulates hMRP2 expression and activity in Caco-2 cells. This hMRP2 induction was estrogen receptor β (ERβ)-mediated but not associated with changes at the mRNA level, thus suggesting a post-transcriptional regulation [90]. The implication of ERβ in such kind of regulation is

possible since it was reported to regulate miRNAs expression [91] which, in turn, can alter hMRP2 expression [92]. Similarly, Caco-2 cells exposed to GNT (1 μM, 48 h) exhibited an ERβ-mediated hMRP2 induction at protein level without changes in expression at the mRNA level. This was associated with increased transporter activity and enhanced protection against 1-chloro-2,4-dinitrobenzene, an MRP2 substrate precursor [90]. GNT can also modulate MRP2 activity in an acute fashion, i.e., without changes in transporter expression. Some years ago, it was reported that this isoflavone competitively inhibits hepatic rMrp2 in isolated and perfused rat liver model [93]. In line with this result, Yokooji et al. demonstrated that GNT administered intravenously reduced rMrp2-mediated secretion of irinotecan hydrochloride and its metabolites in rat liver and intestine [94].

The uricosuric drug probenecid represents another example of xenobiotics affecting intestinal MRP2 activity without modifying MRP2 expression. Probenecid is a classical competitive inhibitor of organic anions transport also used in the clinical practice to enhance plasma levels of antibiotics. Although nonspecific, its inhibitory effect on intestinal MRP2 was clearly demonstrated in rats [95] and in Caco-2 cells [90]. Nowadays, more potent and specific MRP inhibitors like MK571 are preferred for characterization of transport specificity. Finally, various members of the large family of nonsteroidal anti-inflammatory drugs are recognized MRP2 modulators [96]. In intestine, indomethacin was shown to inhibit MRP2 and to increase sulfasalazine transepithelial permeability, both in rat small intestine and in Caco-2 cell monolayers [97]. In agreement with these findings, Caco-2 cells coincubated with indomethacin exhibited an increased permeability to the hMRP2 substrates fluvastatin [98] and colchicine [99].

3. Conclusion

Although the organisms are permanently exposed to a wide range of xenobiotics such as therapeutic drugs, environmental pollutants, and natural toxins, they possess a sophisticate system of detoxification in which metabolizing enzymes and transport proteins play an essential role. Evidence from *in vitro* and *in vivo* studies unambiguously demonstrates that MRP2 is a crucial actor in protecting specific tissues from xenobiotic toxicity. It is noteworthy that MRP2 also plays a crucial role in elimination of endogenous metabolites. A list of relevant and well-recognized substrates of MRP2 is presented in **Table 1**. Two relevant tissues expressing MRP2 are the liver and the small intestine. It should be noted that other participants not contemplated in this review, such as a bunch of metabolizing enzymes as well as members of the family of ABC transporters different from MRP2, are additionally involved in xenobiotic detoxification.

Expression and activity of MRP2 can be modulated at both transcriptional and post-transcriptional levels. The nuclear receptor PXR plays a major role in transcriptional regulations. PXR functions as sensor for many agents and its activation leads to a coordinated response on biotransformation enzymes and transport systems. Examples of these agents are rifampicin, carbamazepine, pregnenolone-16α-carbonitrile, efavirenz, saquinavir, BZL, SL, etc. Other

xenobiotics such as bortezomib, BPA, and sulforaphane regulate MRP2 as a result of the interaction with Nrf2. Alternatively, regulation of MRP2 activity by xenobiotics can occur without changes in transporter expression, as it was demonstrated to GNT. Finally, it is important to note that regulation of MRP2 activity by therapeutic agents can result in changes in their therapeutic efficacy or safety, or alternative in drug–drug interactions if other drugs, substrates of MRP2, are simultaneously administered.

Abbreviations: ABC, ATP-binding cassette; AP-1, activator protein-1; APAP, acetaminophen; ARE, antioxidant response elements; ATF2, activating transcription factor-2; BPA, bisphenol A; BZL, benznidazole; C/EBPβ, CCAAT/enhancer-binding protein-β; cAMP, cyclic adenosine monophosphate; CAR, constitutive androstane receptor; CRE, cAMP response element; CYP, cytochrome P450; DDT, dichlorodiphenyltrichloroethane; DNP-SG, dinitrophenyl-S-glutathione; EE, ethynylestradiol; EHBR, Eisai hyperbilirubinemic rats; ER, erucin; ERβ, estrogen receptor β; FXR, farnesoid X-activated receptor; GNT, genistein; GY, Groningen Yellow; GSH, glutathione; GST, glutathione S-transferase; HNFs, hepatic nuclear factors; IGF-1R, insulin-like growth factor receptor 1; IL-6, interleukin 6; Keap1, Kelch-like ECH-associated protein 1; L0, linker segment 0; LXRα, liver X receptor α; MAPK, mitogen-activated protein kinases; MRP, multidrug resistance-associated protein; MRP2, multidrug resistance-associated protein 2; hMRP2, human MRP2; rMrp2, rat Mrp2; mMrp2, mouse Mrp2; cMrp2, chickenMrp2; MSDs, membrane-spanning domains; MTX, methotrexate; NAPQI, N-acetyl-p-benzoquinone imine; NBDs, nucleotide-binding domains; NO, nitric oxide; Nrf2, nuclear factor-erythroid 2- related factor-2; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; PI3K, phosphoinositide 3-kinase; PPARα, peroxisome proliferator activated receptor alpha; PXR, pregnane X receptor; RARα, retinoic acid receptor α; RES, resveratrol; RXRα, retinoid X receptor α; SF, sulforaphane; SL, spironolactone; SULT, sulphotransferase; TLR4, tool-like receptor 4; UGT, UDP-glucuronosyltransferase.

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References

[1] Suzuki H, Sugiyama Y. Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. Eur J Pharm Sci 2000;12:3–12. doi:10.1016/S0928-0987(00)00178-0.

[2] Liu L, Pang KS. The roles of transporters and enzymes in hepatic drug processing. Drug Metab Dispos 2005;33:1–9. doi:10.1124/dmd.104.001149.

[3] Dietrich CG, Geier a, Oude Elferink RPJ. ABC of oral bioavailability: transporters as gatekeepers in the gut. Gut 2003;52:1788–95. doi:10.1136/gut.52.12.1788.

[4] Jedlitschky G, Hoffmann U, Kroemer H. Structure and function of the MRP2 (ABCC2) protein and its role in drug disposition. Expert Opin Drug Metab Toxicol 2006;2:351–66.

[5] Payen L, Sparfel L, Courtois A, Vernhet L, Guillouzo A, Fardel O. The drug efflux pump MRP2: regulation of expression in physiopathological situations and by endogenous and exogenous compounds. Cell Biol Toxicol 2002;18:221–33. doi:10.1023/A:1016020626941.

[6] Fardel O, Jigorel E, Le Vee M, Payen L. Physiological, pharmacological and clinical features of the multidrug resistance protein 2. Biomed Pharmacother 2005;59:104–14. doi:10.1016/j.biopha.2005.01.005.

[7] Zhang Y, Li W, Vore M. Translational regulation of rat multidrug resistance- associated protein 2 expression is mediated by upstream open reading frames in the 5J untranslated region. Mol Pharmacol 2007;71:377–83. doi:10.1124/mol.106.029793.2002.

[8] Mottino AD, Hoffman T, Jennes L, Vore M. Expression and localization of multidrug resistant protein mrp2 in rat small intestine. J Pharmacol Exp Ther 2000;293:717–23.

[9] Clifton G, Kaplowitz N. The glutathione S-transferases of the small intestine in the rat. Cancer Res 1977;37:788–91.

[10] Shelby MK, Cherrington NJ, Vansell NR, Klaassen CD. Tissue mRNA expression of the rat UDP-glucuronosyltransferase gene family. Drug Metab Dispos 2003;31:326–33. doi:10.1124/dmd.31.3.326.

[11] Keppler D, Leier I, Jedlitschky G. Transport of glutathione conjugates and glucuronides by the multidrug resistance proteins MRP1 and MRP2. Biol Chem 1997;378:787–91.

[12] Keppler D, König J. Hepatic secretion of conjugated drugs and endogenous substances. Semin Liver Dis 2000;20:265–72. doi:10.1055/s-2000-9391.

[13] Paulusma C, Kothe M, Bakker C, Bosma P, van Bokhoven I, van Marle J, et al. Zonal down-regulation and redistribution of the multidrug resistance protein 2 during bile duct ligation in rat liver. Hepatology 2000;31:684–93.

[14] Chowdhury JR, Novikoff PM, Chowdhury NR, Novikoff AB. Distribution of UDPglucuronosyltransferase in rat tissue. Proc Natl Acad Sci U S A 1985;82:2990–4.

[15] Jansen PL, Peters WH, Lamers WH. Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport. Hepatology 1985;5:573–9. doi:10.1002/hep.1840050408.

[16] Huber M, Guhlmann A, Jansen P, Keppler D. Hereditary defect of hepatobiliary cysteinyl leukotriene elimination in mutant rats with defective hepatic anion excretion. Hepatology 1987;7:224–8.

[17] Takenaka O, Horie T, Kobayashi K, Suzuki H, Sugiyama Y. Kinetic analysis of hepatobiliary transport for conjugated metabolites in the perfused liver of mutant rats (EHBR) with hereditary conjugated hyperbilirubinemia. Pharm Res 1995;12:1746–55.

[18] Vaidyanathan JB, Walle T. Transport and metabolism of the tea flavonoid (-)-epicatechin by the human intestinal cell line Caco-2. Pharm Res 2001;18:1420–5. doi:10.1023/A:1012200805593.

[19] Walle UK, Galijatovic A, Walle T. Transport of the flavonoid chrysin and its conjugated metabolites by the human intestinal cell line Caco-2. Biochem Pharmacol 1999;58:431–8. doi:10.1016/S0006-2952(99)00133-1.

[20] Dietrich CG, De Waart DR, Ottenhoff R, Bootsma AH, van Gennip AH, Elferink RP. Mrp2-deficiency in the rat impairs biliary and intestinal excretion and influences metabolism and disposition of the food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo. Carcinogenesis 2001;22:805–11. doi:10.1093/carcin/22.5.805.

[21] Kauffmann HM, Keppler D, Gant TW, Schrenk D. Induction of hepatic mrp2 (cmrp/cmoat) gene expression in nonhuman primates treated with rifampicin or tamoxifen. Arch Toxicol 1998;72:763–8. doi:10.1007/s002040050571.

[22] Gerk PM, Vore M. Regulation of expression of the multidrug resistance- associated protein 2 (MRP2) and its role in drug disposition. J Pharmacol Exp Ther 2002;302:407–15. doi:10.1124/jpet.102.035014.chemotherapeutic.

[23] Kauffmann HM, Schrenk D. Sequence analysis and functional characterization of the 5′-flanking region of the rat multidrug resistance protein 2 (mrp2) gene. Biochem Biophys Res Commun 1998;245:325–31. doi:10.1006/bbrc.1998.8340.

[24] Stöckel B, König J, Nies a T, Cui Y, Brom M, Keppler D. Characterization of the 5′-flanking region of the human multidrug resistance protein 2 (MRP2) gene and its regulation in comparison with the multidrug resistance protein 3 (MRP3) gene. Eur J Biochem 2000;267:1347–58.

[25] Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, et al. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. J Biol Chem 2002;277:2908–15. doi:10.1074/jbc.M109326200.

[26] Arana MR, Tocchetti GN, Domizi P, Arias A, Rigalli JP, Ruiz ML, et al. Coordinated induction of GST and MRP2 by cAMP in Caco-2 cells: role of protein kinase A signaling pathway and toxicological relevance. Toxicol Appl Pharmacol 2015;287:178–90. doi: 10.1016/j.taap.2015.06.003.

[27] Häussinger D, Schmitt M, Weiergraber O, Kubitz R. Short-term regulation of canalicular transport. Semin Liver Dis 2000;20:307–21.

[28] Mottino AD, Cao J, Veggi LM, Crocenzi F, Roma MG, Vore M. Altered localization and activity of canalicular Mrp2 in estradiol-17B-D-glucuronide-induced cholestasis. Hepatology 2002;35:1409–19. doi:10.1053/jhep.2002.33327.

[29] Roelofsen H, Soroka CJ, Keppler D, Boyer JL. Cyclic AMP stimulates sorting of the canalicular organic anion transporter (Mrp2/cMoat) to the apical domain in hepatocyte couplets. J Cell Sci 1998;111:1137–45.

[30] Paulusma CC, Kool M, Bosma PJ, Scheffer GL, Borg F Ter, Scheper RJ, et al. A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin-Johnson syndrome. Hepatology 1997;25:1539–42. doi:10.1002/hep.510250635.

[31] Mor-Cohen R, Zivelin a, Rosenberg N, Goldberg I, Seligsohn U. A novel ancestral splicing mutation in the multidrug resistance protein 2 gene causes Dubin-Johnson syndrome in Ashkenazi Jewish patients. Hepatol Res 2005;31:104–11. doi:S1386-6346(04)00330-4 [pii]\r10.1016/j.hepres.2004.11.010.

[32] Jones BR, Li W, Cao J, Hoffman TA, Gerk PM, Vore M. The role of protein synthesis and degradation in the post-transcriptional regulation of rat multidrug resistance- associated protein 2 (Mrp2, Abcc2). Mol Pharmacol 2005;68:701–10. doi:10.1124/mol.105.013144.independent.

[33] Zhang Y, Zhao T, Li W, Vore M. The 5′-untranslated region of multidrug resistance associated protein 2 (MRP2; ABCC2) regulates downstream open reading frame expression through translational regulation. Mol Pharmacol 2010;77:237–46. doi: 10.1124/mol.109.058982.

[34] di Masi A, Marinis E De, Ascenzi P, Marino M. Nuclear receptors CAR and PXR: molecular, functional, and biomedical aspects. Mol Aspects Med 2009;30:297–343. doi: 10.1016/j.mam.2009.04.002.

[35] Fujiyama N, Shitara Y, Horie T. The mechanism of the down-regulation of hepatic transporters in rats with indomethacin-induced intestinal injury. Dig Dis Sci 2013;58:1891–8. doi:10.1007/s10620-013-2587-z.

[36] Cherrington NJ, Slitt AL, Li N, Klaassen CD. Lipopolysaccharide-mediated regulation of hepatic transporter mRNA levels in rats. Drug Metab Dispos 2004;32:734–41. doi: 10.1124/dmd.32.7.734.

[37] Fang C, Yoon S, Tindberg N, Järveläinen HA, Lindros KO, Ingelman-Sundberg M. Hepatic expression of multiple acute phase proteins and down-regulation of nuclear

receptors after acute endotoxin exposure. Biochem Pharmacol 2004;67:1389–97. doi: 10.1016/j.bcp.2003.12.012.

[38] Masubuchi Y, Masuda E, Horie T. Multiple mechanisms in indomethacin-induced impairment of hepatic cytochrome P450 enzymes in rats. Gastroenterology 2002;122:774–83. doi:10.1053/gast.2002.31886.

[39] El Kasmi K, Anderson A, Devereaux M, Vue P, Zhang W, Setchell K, et al. Phytosterols promote liver injury and Kupffer cell activation in parenteral nutrition-associated liver disease. Sci Transl Med 2013;5:206ra137. doi:10.1038/jid.2014.371.

[40] Ruiz ML, Villanueva SS, Luquita MG, Sánchez-Pozzi EJ, Crocenzi FA, Pellegrino JM, et al. Mechanisms involved in spironolactone-induced choleresis in the rat: role of multidrug resistance-associated protein 2. Biochem Pharmacol 2005;69:531–9. doi: 10.1016/j.bcp.2004.10.017.

[41] Miguet J, Vuitton D, Thebault-Lucas A, Joanne C, Dhumeaux D. Spironolactone and enzyme induction in patients with alcoholic cirrhosis. Gastroenterology 1980;78:996–1000.

[42] Wirth KE, Frölich JC, Holliffield JW, Falkner FC, Sweetman BS, Oates JA. Metabolism of digitoxin in man and its modification by spironolactone. Eur J Clin Pharmacol 1976;9:345–54.

[43] Perdomo VG, Rigalli JP, Villanueva SSM, Ruiz ML, Luquita MG, Echenique CG, et al. Modulation of biotransformation systems and ABC transporters by benznidazole in rats. Antimicrob Agents Chemother 2013;57:4894–902. doi:10.1128/AAC.02531-12.

[44] Rigalli JP, Perdomo VG, Luquita MG, Villanueva SSM, Arias A, Theile D, et al. Regulation of biotransformation systems and abc transporters by benznidazole in HepG2 cells: involvement of pregnane X-receptor. PLoS Negl Trop Dis 2012;6. doi:10.1371/journal.pntd.0001951.

[45] Zhou L, Song Y, Zhao J, Qin H, Zhang G, Zhou Y, et al. Monoammonium glycyrrhizinate protects rifampicin- and isoniazid-induced hepatotoxicity via regulating the expression of transporter Mrp2, Ntcp, and Oatp1a4 in liver. Pharm Biol 2016;54:931–7. doi: 10.3109/13880209.2015.1070878.

[46] McGill M, Jaeschke H. Recent advances in relation to hepatotoxicity and diagnosis. Pharm Res 2014;30:2174–87. doi:10.1007/s11095-013-1007-6.METABOLISM.

[47] Ghanem CI, Gómez PC, Arana MC, Perassolo M, Ruiz ML, Villanueva SSM, et al. Effect of acetaminophen on expression and activity of rat liver multidrug resistance-associated protein 2 and P-glycoprotein. Biochem Pharmacol 2004;68:791–8. doi:10.1016/j.bcp.2004.05.014.

[48] Aleksunes LM, Scheffer GL, Jakowski AB, Pruimboom-Brees IM, Manautou JE. Coordinated expression of multidrug resistance-associated proteins (Mrps) in mouse

liver during toxicant-induced injury. Toxicol Sci 2006;89:370–9. doi:10.1093/toxsci/kfi332.

[49] Maher JM, Dieter MZ, Aleksunes LM, Slitt AL, Guo G, Tanaka Y, et al. Oxidative and electrophilic stress induces multidrug resistance-associated protein transporters via the nuclear factor-E2-related factor-2 transcriptional pathway. Hepatology 2007;46:1597–610. doi:10.1002/hep.21831.

[50] Wang W, Guan C, Sun X, Zhao Z, Li J, Fu X, et al. Tanshinone IIA protects against acetaminophen-induced hepatotoxicity via activating the Nrf2 pathway. Phytomedicine 2016;23:589–96. doi:10.1016/j.phymed.2016.02.022.

[51] Jiang Y, Wang Y, Tan H, Yu T, Fan X, Chen P, et al. Schisandrol B protects against acetaminophen-induced acute hepatotoxicity in mice via activation of the NRF2/ARE signaling pathway. Acta Pharmacol Sin 2016;37:382–9. doi:10.1038/aps.2015.120.

[52] Noh JR, Kim YH, Hwang JH, Choi DH, Kim KS, Oh WK, et al. Sulforaphane protects against acetaminophen-induced hepatotoxicity. Food Chem Toxicol 2015;80:193–200. doi:10.1016/j.fct.2015.03.020.

[53] Battaille A, Manautou JE. Nrf2: a potential target for new therapeutics in liver disease. Clin Pharmacol Ther 2012;92:340–8. doi:10.1016/j.biotechadv.2011.08.021.Secreted.

[54] Vollrath V, Wielandt AM, Iruretagoyena M, Chianale J. Role of Nrf2 in the regulation of the Mrp2 (ABCC2) gene. Biochem J 2006;395:599–609. doi:10.1042/BJ20051518.

[55] Wu Z-T, Qi X-M, Sheng J-J, Ma L-L, Ni X, Ren J, et al. Timosaponin A3 induces hepatotoxicity in rats through inducing oxidative stress and down-regulating bile acid transporters. Acta Pharmacol Sin 2014;35:1188–98. doi:10.1038/aps.2014.65.

[56] Li G xing, Pei Q ling, Gao Y, Liu K ming, Nie J sheng, Han G, et al. Protective effects of hepatocellular canalicular conjugate export pump (Mrp2) on sodium arsenite-induced hepatic dysfunction in rats. Exp Toxicol Pathol 2007;58:447–53. doi:10.1016/j.etp.2007.02.001.

[57] Liu B, Pan S, Dong X, Qiao H, Jiang H, Krissansen GW, et al. Opposing effects of arsenic trioxide on hepatocellular carcinomas in mice. Cancer Sci 2006;97:675–81. doi:10.1111/j.1349-7006.2006.00230.x.

[58] Osselaere A, Li SJ, De Bock L, Devreese M, Goossens J, Vandenbroucke V, et al. Toxic effects of dietary exposure to T-2 toxin on intestinal and hepatic biotransformation enzymes and drug transporter systems in broiler chickens. Food Chem Toxicol 2013;55:150–5. doi:10.1016/j.fct.2012.12.055.

[59] Bucher S, Le Vee M, Jouan E, Fardel O. Regulation of hepatic drug transporter activity and expression by organochlorine pesticides. J Biochem Mol Toxicol 2014;28:119–28. doi:10.1002/jbt.

[60] Coumoul X, Diry M, Barouki R. PXR-dependent induction of human CYP3A4 gene expression by organochlorine pesticides. Biochem Pharmacol 2002;64:1513–9. doi: 10.1016/S0006-2952(02)01298-4.

[61] Chepelev NL, Enikanolaiye MI, Chepelev LL, Almohaisen A, Chen Q, Scoggan KA, et al. Bisphenol A activates the Nrf1/2-antioxidant response element pathway in HEK 293 cells. Chem Res Toxicol 2013;26:498–506. doi:10.1021/tx400036v.

[62] Moscovitz JE, Nahar MS, Shalat SL, Slitt AL, Dolinoy DC, Aleksunes LM. Correlation between conjugated bisphenol A concentrations and efflux transporter expression in human fetal livers. Drug Metab Dispos 2016;44:1061–5. doi:10.1124/dmd.115.068668.

[63] Fromm MF, Kauffmann HM, Fritz P, Burk O, Kroemer HK, Warzok RW, et al. The effect of rifampin treatment on intestinal expression of human MRP transporters. Am J Pathol 2000;157:1575–80. doi:10.1016/S0002-9440(10)64794-3.

[64] Oswald S, Haenisch S, Fricke C, Sudhop T, Remmler C, Giessmann T, et al. Intestinal expression of P-glycoprotein (ABCB1), multidrug resistance associated protein 2 (ABCC2), and uridine diphosphate-glucuronosyltransferase 1A1 predicts the disposition and modulates the effects of the cholesterol absorption inhibitor ezetimibe in. Clin Pharmacol Ther 2006;79:206–17. doi:10.1016/j.clpt.2005.11.004.

[65] König SK, Herzog M, Theile D, Zembruski N, Haefeli WE, Weiss J. Impact of drug transporters on cellular resistance towards saquinavir and darunavir. J Antimicrob Chemother 2010;65:2319–28. doi:10.1093/jac/dkq324.

[66] Luo G, Cunningham M, Kim S, Burn T, Lin J, Sinz M, et al. CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. Drug Metab Dispos 2002;30:795–804. doi:10.1124/dmd.30.7.795.

[67] Giessmann T, May K, Modess C, Wegner D, Hecker U, Zschiesche M, et al. Carbamazepine regulates intestinal P-glycoprotein and multidrug resistance protein MRP2 and influences disposition of talinolol in humans. Clin Pharmacol Ther 2004;76:192–200. doi:10.1016/j.clpt.2004.04.011.

[68] Cheng X, Klaassen CD. Regulation of mRNA expression of xenobiotic transporters by the pregnane X receptor in mouse liver, kidney, and intestine. Drug Metab Dispos 2006;34:1863–7. doi:10.1124/dmd.106.010520.

[69] Sharma D, Lau AJ, Sherman MA, Chang TKH. Agonism of human pregnane X receptor by rilpivirine and etravirine: comparison with first generation non-nucleoside reverse transcriptase inhibitors. Biochem Pharmacol 2013;85:1700–11. doi:10.1016/j.bcp.2013.04.002.

[70] Weiss J, Herzog M, König S, Storch CH, Ketabi-Kiyanvash N, Haefeli WE. Induction of multiple drug transporters by efavirenz. J Pharmacol Sci 2009;109:242–50. doi:10.1254/jphs.08209FP.

[71] Weiss J, Baumann S, Theile D, Haefeli WE. Desmethyl bosentan displays a similar *in vitro* interaction profile as bosentan. Pulm Pharmacol Ther 2015;30:80–6. doi:10.1016/j.pupt.2014.12.001.

[72] Theile D, Haefeli WE, Weiss J. Effects of adrenolytic mitotane on drug elimination pathways assessed *in vitro*. Endocrine 2015;49:842–53. doi:10.1007/s12020-014-0517-2.

[73] Ruiz ML, Villanueva SSM, Luquita MG, Pellegrino JM, Rigalli JP, Arias A, et al. Induction of intestinal multidrug resistance-associated protein 2 (Mrp2) by spironolactone in rats. Eur J Pharmacol 2009;623:103–6. doi:10.1016/j.ejphar.2009.09.014.

[74] Haslam IS, Jones K, Coleman T, Simmons NL. Induction of P-glycoprotein expression and function in human intestinal epithelial cells (T84). Biochem Pharmacol 2008;76:850–61. doi:10.1016/j.bcp.2008.07.020.

[75] Martin P, Riley R, Back DJ, Owen A. Comparison of the induction profile for drug disposition proteins by typical nuclear receptor activators in human hepatic and intestinal cells. Br J Pharmacol 2008;153:805–19. doi:10.1038/sj.bjp.0707601.

[76] Theile D, Allendorf D, Köhler B, Jassowicz A, Weiss J. Obatoclax as a perpetrator in drug-drug interactions and its efficacy in multidrug resistance cell lines. J Pharm Pharmacol 2015;67:1575–84. doi:10.1371/journal.pone.0136080.

[77] Ebert B, Kisiela M, Wsól V, Maser E. Proteasome inhibitors MG-132 and bortezomib induce AKR1C1, AKR1C3, AKR1B1, and AKR1B10 in human colon cancer cell lines SW-480 and HT-29. Chem Biol Interact 2011;191:239–49. doi:10.1016/j.cbi.2010.12.026.

[78] Aleksunes LM, Goedken MJ, Rockwell CE, Thomale J, Manautou JE, Klaassen CD. Transcriptional regulation of renal cytoprotective genes by Nrf2 and its potential use as a therapeutic target to mitigate cisplatin-induced nephrotoxicity. J Pharmacol Exp Ther 2010;335:2–12. doi:10.1124/jpet.110.170084.

[79] Ghanem CI, Rudraiah S, Bataille AM, Vigo MB, Goedken MJ, Manautou JE. Role of nuclear factor-erythroid 2-related factor 2 (Nrf2) in the transcriptional regulation of brain ABC transporters during acute acetaminophen (APAP) intoxication in mice. Biochem Pharmacol 2015;94:203–11. doi:10.1016/j.bcp.2015.01.013.

[80] Sestili P, Fimognari C. Cytotoxic and antitumor activity of sulforaphane: the role of reactive oxygen species. Biomed Res Int 2015; 2015:1–9.

[81] Traka M, Gasper AV., Smith JA, Hawkey CJ, Bao Y, Mithen RF. Transcriptome analysis of human colon Caco-2 cells exposed to sulforaphane. J Nutr 2005;135:1865–72.

[82] Jakubíková J, Sedlák J, Mithen R, Bao Y. Role of PI3K/Akt and MEK/ERK signaling pathways in sulforaphane- and erucin-induced phase II enzymes and MRP2 transcription, G2/M arrest and cell death in Caco-2 cells. Biochem Pharmacol 2005;69:1543–52. doi:10.1016/j.bcp.2005.03.015.

[83] Harris KE, Jeffery EH. Sulforaphane and erucin increase MRP1 and MRP2 in human carcinoma cell lines. J Nutr Biochem 2008;19:246–54. doi:10.1016/j.jnutbio.2007.02.014.

[84] Wang X, Campos CR, Peart JC, Smith LK, Boni JL, Cannon RE, et al. Nrf2 up-regulates ATP binding cassette transporter expression and activity at the blood-brain and blood-spinal cord barriers. J Neurosci 2014;34:8585–93. doi:10.1523/JNEUROSCI.2935-13.2014.

[85] Bock KW, Eckle T, Ouzzine M, Fournel-Gigleux S. Coordinate induction by antioxidants of UDP-glucuronosyltransferase UGT1A6 and the apical conjugate export pump MRP2 (multidrug resistance protein 2) in Caco-2 cells. Biochem Pharmacol 2000;59:467–70. doi:10.1016/S0006-2952(99)00366-4.

[86] Jia Y, Liu Z, Huo X, Wang C, Meng Q, Liu Q, et al. Enhancement effect of resveratrol on the intestinal absorption of bestatin by regulating PEPT1, MDR1 and MRP2 in vivo and *in vitro*. Int J Pharm 2015;495:588–98. doi:10.1016/j.ijpharm.2015.09.042.

[87] Sane RS, Steinmann GG, Huang Q, Li Y, Podila L, Mease K, et al. Mechanisms underlying benign and reversible unconjugated hyperbilirubinemia observed with faldaprevir administration in hepatitis C virus patients. J Pharmacol Exp Ther 2014;351:403–12. doi:10.1124/jpet.114.218081.

[88] Kiser JJ, Burton Jr JR, Everson GT. Drug-drug interactions during antiviral therapy for chronic hepatitis C. Nat Rev Gastroenterol Hepatol 2013;10:596–606. doi:10.1038/nrgastro.2013.106.Drug.

[89] Arias A, Villanueva SSM, Ruiz ML, Luquita MG, Veggi LM, Pellegrino M, et al. Regulation of expression and activity of rat intestinal multidrug resistance-associated protein 2 by cholestatic estrogens. Drug Metab Dispos 2009;37:1277–85. doi:10.1124/dmd.108.025643.thus.

[90] Arias A, Rigalli JP, Villanueva SSM, Ruiz ML, Luquita MG, Perdomo VG, et al. Regulation of expression and activity of multidrug resistance proteins MRP2 and MDR1 by estrogenic compounds in Caco-2 cells. Role in prevention of xenobiotic-induced cytotoxicity. Toxicology 2014;320:46–55. doi:10.1016/j.tox.2014.03.007.

[91] Edvardsson K, Nguyen-Vu T, Kalasekar SM, Pontén F, Gustafsson J-A, Williams C. Estrogen receptor B expression induces changes in the microRNA pool in human colon cancer cells. Carcinogenesis 2013;34:1431–41. doi:10.1093/carcin/bgt067.

[92] Haenisch S, Laechelt S, Bruckmueller H, Werk A, Noack A, Bruhn O, et al. Down-regulation of ATP-binding cassette C2 protein expression in HepG2 cells after rifampicin treatment is mediated by microRNA-379. Mol Pharmacol 2011;80:314–20. doi:10.1124/mol.110.070714.

[93] Jäger W, Winter O, Halper B, Salamon A, Sartori M, Gajdzik L, et al. Modulation of liver canalicular transport processes by the tyrosine-kinase inhibitor genistein: implications

of genistein metabolism in the rat. Hepatology 1997;26:1467–76. doi:10.1053/jhep.1997.v26.pm0009397986.

[94] Yokooji T, Kawabe Y, Mori N, Murakami T. Effect of genistein, a natural soy isoflavone, on the pharmacokinetics and intestinal toxicity of irinotecan hydrochloride in rats. J Pharm Pharmacol 2013;65:280–91. doi:10.1111/j.2042-7158.2012.01592.x.

[95] Naruhashi K, Tamai I, Inoue N, Muraoka H, Sai Y, Suzuki N, et al. Involvement of multidrug resistance-associated protein 2 in intestinal secretion of grepafloxacin in rats. Antimicrob Agents Chemother 2002;46:344–9. doi:10.1128/AAC.46.2.344-349.2002.

[96] El-Sheikh AAK, van den Heuvel JJMW, Koenderink JB, Russel FGM. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/ABCC2-and MRP4/ABCC4-mediated methotrexate transport. J Pharmacol Exp Ther 2007;320:229–35. doi:10.1124/jpet.106.110379.ceiving.

[97] Dahan A, Amidon GL. MRP2 mediated drug-drug interaction: indomethacin increases sulfasalazine absorption in the small intestine, potentially decreasing its colonic targeting. Int J Pharm 2010;386:216–20. doi:10.1016/j.ijpharm.2009.11.021.

[98] Lindahl A, Sjöberg A, Bredberg U, Toreson H, Ungell A-L, Lennernäs H. Regional intestinal absorption and biliary excretion of fluvastatin in the rat: possible involvement of mrp2. Mol Pharm 2004;1:347–56. doi:10.1021/mp0499297.

[99] Dahan A, Sabit H, Amidon GL. Multiple efflux pumps are involved in the transepithelial transport of colchicine: Combined effect of P-glycoprotein and multidrug resistance- associated protein 2 leads to decreased intestinal absorption throughout the entire small intestine. Drug Metab Dispos 2009;37:2028–36. doi:10.1124/dmd.109.028282.

Chapter 3

Nanotoxicology: A Review

Chavon Walters, Edmund Pool and
Vernon Somerset

Additional information is available at the end of the chapter

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Abstract

Nanotoxicology represents a new and growing research area in toxicology. It deals with the assessment of the toxicological properties of nanoparticles (NPs) with the intention of determining whether (and to what extent) they pose an environmental or societal threat. Inherent properties of NPs (including size, shape, surface area, surface charge, crystal structure, coating, and solubility/dissolution) as well as environmental factors (such as temperature, pH, ionic strength, salinity, and organic matter) collectively influence NP behavior, fate and transport, and ultimately toxicity. The mechanisms underlying the toxicity of nanomaterials (NMs) have recently been studied extensively. Reactive oxygen species (ROS) toxicity represents one such mechanism. An overproduction of ROS induces oxidative stress, resulting in inability of the cells to maintain normal physiological redox-regulated functions. In the context of this book, this chapter includes topics pertaining to chemical and physical properties of NMs and characterization for proper toxicological evaluation, exposure, and environmental fate and transport, and ecological and genotoxic effects. This chapter reviews the available research pertaining specifically to NMs in the aquatic environment (in plants, aquatic invertebrates, and fish) and their use in biomarker studies.

Keywords: nanomaterials, characterization techniques, nanotoxicology, biomarkers, analytical methods, in vitro studies, in vivo studies

1. Introduction

Nanotoxicology is regarded as the assessment of the toxicological properties of nanoparticles (NPs) with the intention of determining whether (and to what extent) they may pose an environmental or societal threat. Nanotechnology has advanced exponentially over the past

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decade, with nanoscale materials being exploited in several applications and in several disciplines (including industry, science, pharmacy, medicine, electronics, and communication products). Vance et al. [1] reported a 30-fold increase in nano-based products between 2011 and 2015 (**Figure 1**) and an estimated global market of over $1 trillion in 2015 [2]. Metal NPs (specifically, carbon and silver NPs) represent the largest and fastest growing group of NPs (**Figure 2**). Hence, human and environmental exposure is already occurring and is predicted to increase dramatically. This growth in nanotechnology has not advanced without concerns regarding their potential adverse environmental impacts. Several reviews have reported on the toxicity of various NPs [3, 4]. However, much is still unknown.

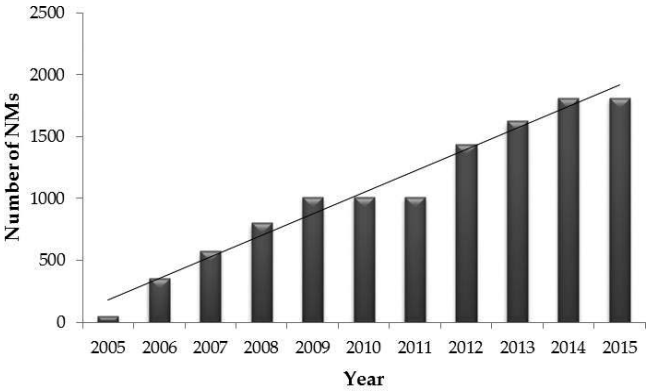

Figure 1. Nanomaterial growth trend 2010–2015 [1].

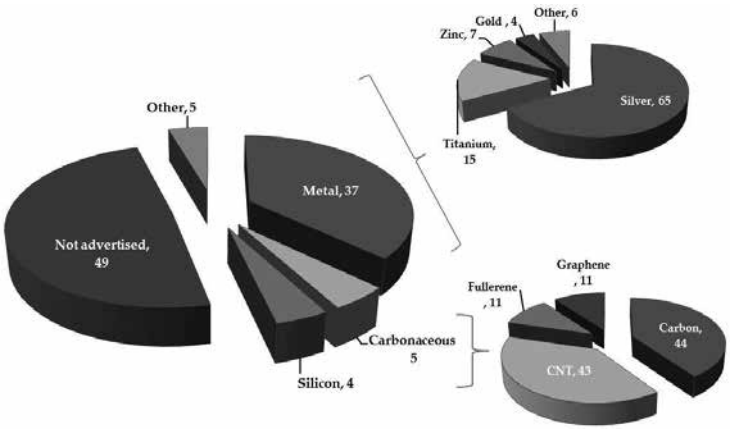

Figure 2. Composition of nanomaterials (adapted from Vance et al. [1]).

Nanomaterials (NMs) are generally defined as a substance having particles with at least one dimension of 1–100 nm in length. Their novel physical and chemical characteristics have made them useful in several applications; however, these very properties can be potentially toxic.

Once introduce into aquatic ecosystems, the fate and toxicity of NPs and its uptake by aquatic organisms depend on several factors. Both the properties of NP (such as size, shape, and coatings) and water chemistry (such as dissolved organic carbon, ionic strength, pH, temperature) will largely influence the extent to which NPs will either remain in suspension, partition to dissolved organic carbon in the water column, form aggregates, or adsorb to suspended particles. In aquatic organisms, the accumulation of NPs is dependent on both the uptake and the elimination of the NP out of the organism. These processes also regulate the bioaccumulation (and bioavailability) of NPs. The availability of appropriate methodologies is needed to address key issues in nanotoxicology and to gain a better understanding of nanoparticle toxicity mechanisms (including oxidative stress, cytotoxicity, genotoxicity, and inflammatory responses). Exposure to NMs is largely through ingestion and adsorption to surface epithelia such as the gills.

Aquatic ecosystems are progressively coming under pressure due to the presence of emerging anthropogenic contaminants, including NMs, posing health hazards to inhabitant organisms. In recent years, increasing data demonstrated that NPs could induce toxicity and genotoxicity under a variety of exposure scenarios. An accepted mechanism by which NPs may induce cytotoxicity is considered to be through the induction of reactive oxygen species (ROS) which can induce oxidative stress which in turn may lead to cytotoxicity, DNA damage, and other effects.

Although research on the environmental impacts of NMs has grown dramatically over the past decade, studies investigating the environmental fate, transport, and toxicity of a variety of nanomaterials are still lacking. This chapter will review the available research pertaining specifically to NMs in the aquatic environment (in plants, aquatic invertebrates, and fish) and their use in biomarkers studies.

2. Physical and chemical properties of NMs influencing their toxicity

The behavior of NPs in various environmental matrices is complex and involves several processes. Properties of NMs are unique and different from conventional materials. Properties such as (1) particle size, (2) surface area and charge, (3) shape/structure, (4) solubility, and (5) surface coatings are known to affect NP toxicity.

Small owing to their small size, NMs have unique physical and chemical characteristics such as magnetic, optical, thermal, mechanical, electrical properties which make them suitable in several applications including in medicine, electronics, and energy production, and in several consumer products. However, these very properties have the potential to affect humans and the environment adversely. NPs can easily penetrate cell membranes and other biological barriers into living organisms causing cell damage. Studies reporting increased toxicity of NPs when compared to their larger bulk particles have led to a generally assumed hypothesis that NPs are more potent in causing damage. Lankvel et al. [5] reported the significance of particle size of AgNPs, reporting size-specific tissue distribution and size-specific toxicity. Scown et al. [6] reported the lowest aggregation potential for the smallest AgNPs (i.e., 10 nm vs. 35 and

600–1600 nm) and was most highly concentrated in the gills and liver. Gaiser et al. [7] studied the acute and chronic toxicities of nano- and bulk Ag and CeO_2. Reported mortality rates for Ag and AgNP were as follows: micro-Ag at 0.1 mg/L was 13% and at 1 mg/L was 80%, while for AgNP at 0.1 mg/L was 57% and at 1 mg/L was 100%.

Although NPs size can be the most distinguishing characteristic when compared to conventional particles, shape and morphology also represent important factors when considering NP toxicity. Morphology (particles, spheres, rods, cubes, truncated triangles, wires, films, and coatings) affects NP kinetics and their transport in the environment. NP shape is also of importance as triangular nanoplates was reported to have greater inhibition of *Escherichia coli* than spherical- or rod-shaped AgNPs or Ag^+ which can be attributed to the increased reactive, high atom density of the triangular nanoplates [8, 9].

When NPs are discarded, they can enter the aquatic environment as aggregates and soluble ions, which can be highly toxic to aquatic organisms. Aggregation and dissolution are key processes governing NP behavior and toxicity in the aquatic environment. These processes are largely driven by size and surface properties of NMs, as well as by the stability of natural colloids (such as dissolved organic matter). Colloidal stability is affected by several factors including the type environmental conditions such pH, temperature, and ionic strength. Romer et al. [10], investigating the stability of AgNPs, reported rapid aggregation in media with high ionic strength. Similarly, Walters et al. [11] reported higher toxicity due to the formation of smaller aggregates at elevated temperatures. These and other studies reported changes in organism exposure levels and consequent toxicity due to levels of aggregation [12]. Dissolution of NPs is also a significant process determining NPs effects in the aquatic environment. Most NPs do not dissolve in solution, but form colloid dispersions which will either remain dispersed or aggregate. As such, interactions with other colloid materials will affect the rate at which particles aggregate in an aqueous environment.

In the natural environment, NPs are not present in isolation. As such, it is important to consider the presence of other environmental stressors. For example, Walters et al. [13] reported that higher temperatures resulted in higher toxicity due to the formation of smaller aggregates at elevated temperatures and that AgNP dissolution and sedimentation contributed to a higher availability and toxicity of AgNP (and Ag^+) to *Potamonautes perlatus*. Similarly, Liu and Hurt [14] reported higher dissolution rates of AgNPs with increased temperature. In the presence of dissolved oxygen (DO), AgNPs tend to aggregate and release Agions which in turns induce aggregation and oxidation [12].

Surface charge is a major factor in determining the particle dispersion characteristics and also will influence the adsorption of ions and biomolecules [15]. Baalousha [16] reported disaggregation of FeO NPs due to enhanced surface charge. Similarly, El Badawy et al. [17] reported surface charge-dependent toxicity of AgNPs. In addition, surface coating is indirectly related to aggregation and dissolution, as it is reported to increase the surface charge.

These unique physical and chemical properties of NPs raise concern as the conventional assumptions of chemical reactivity and behavior may not necessarily apply with regard to NPs.

3. Characterization

Nanomaterial behavior and toxicity are influenced by their physical and chemical properties. As such, characterization of NMs is essential in order to understand how their physical and chemical properties correlate with chemical, ecological, or biological responses. Full characterization of NPs includes determining the bulk (shape, size, phase, electronic structure, and crystallinity) and surface (surface area, arrangement of surface atoms, surface electronic structure, surface composition, and functionality) properties of the NM. In addition, environmental factors (such as temperature, pH, ionic strength, salinity, organic matter) may also affect NP behavior and toxicity.

Standardized tests established by the Organization for Economic Co-operation and Development (OECD) summarized some of the analytical methods commonly used in the characterization of NMs (OECD ENV/JM/MONO (2016)2. The morphology of NMs is frequently characterized using scanning electron microscopy (SEM) or transmission electron microscopy (TEM). Energy-dispersive X-ray (EDX) spectrometry coupled to SEM is a common method to characterize elemental analysis of NMs. Particle size in aqueous phase may also be determined indirectly by dynamic light scattering (DLS) which measures the Brownian movement of the NPs, or by electrophoretic light scattering spectroscopy (ELS) which uses oscillating electric field. X-ray powder diffraction (XRD) is employed to measure particle size in the dry state and which applies the Scherrer method. Murdock et al. [18] investigated the use of DLS to characterize NM dispersion.

Brunauer-Emmet-Teller (BET) is used to measure surface area. Zeta potential measures surface charge in particles in the aqueous phase and is a fundamental parameters known to affect stability. Atomic force microscopy (AFM) and scanning tunneling microscopy (STM) allow three-dimensional imaging of nanometer scale surfaces and the measurement of forces between surfaces at the pico newton scale. UV-vis and Fourier transform infrared spectroscopy (FTIR) are spectroscopic techniques used in the characterization of fullerenes in solution [19, 20]. Surface enhanced Raman spectroscopy (SERS) is a surface-sensitive technique that enhances Raman scattering by nanostructures allowing the detection of single molecules. Chemical characterization techniques include Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and Energy-dispersive X-ray spectroscopy (EDS) used to investigate the elemental composition of NMs.

4. Routes of exposure in the aquatic environment

Due to the surge in nanotechnology, there have been significant increases in the number of various NPs released into the aquatic environment. **Figure 3** provides a summary of the possible routes in a typical aquatic environment to nanoparticles, potential interactions, and the possible clearance routes. Aquatic ecosystems are susceptible to environmental contamination since they are at the receiving end of contaminants, particularly from runoff sources.

Identified sources of NPs in the aquatic environment include production facilities, production processes, wastewater treatment plants, and accidents during the transport. In addition, aquatic ecosystems are known to sequester and transport contaminants, including NMs. Baun et al. showed that NPs may adhere to algae which may then be consumed by filter-feeders and transfer to higher trophic levels. In the aquatic environment, NPs may aggregate thus reducing the NPs available for direct uptake in the aqueous phase by aquatic organisms. However, aggregated NPs may settle into sediment thereby posing a threat to benthic organisms. In the aquatic environment, NMs are generally associated with sediments [21]. Sediments and soil represent porous environmental matrices which typically have large specific surface areas.

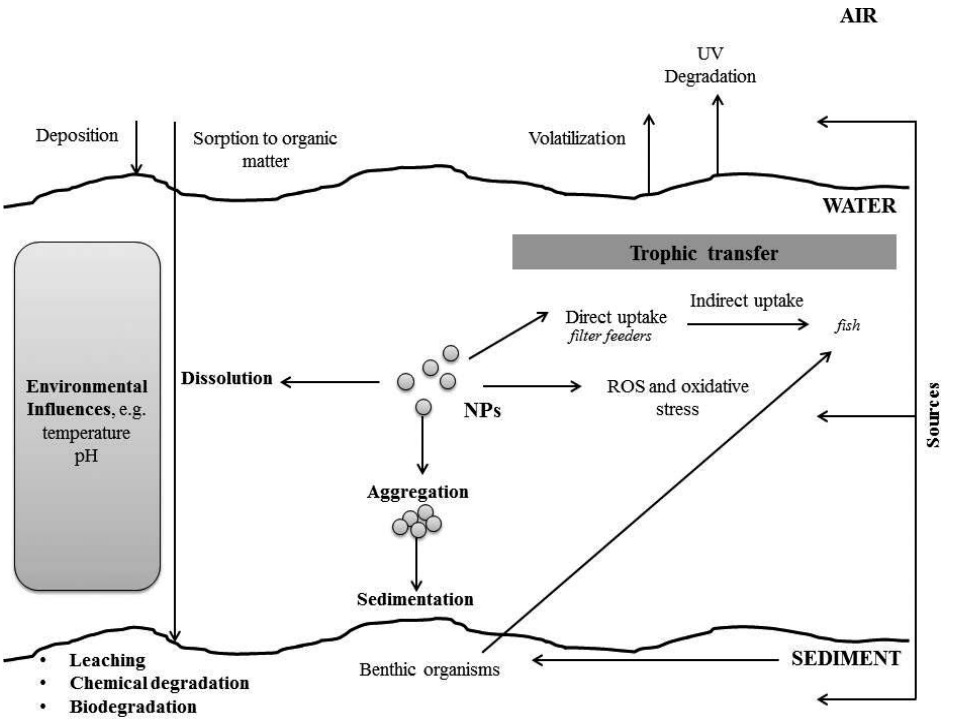

Figure 3. Possible pathways of nanoparticles in the aquatic environment.

Uptake of nanoparticles into the aquatic biota is a major concern. Nanomaterials are able to cross biological barriers, gaining entering due to their small size. In aquatic organisms, the major routes of entry are via ingestion or direct passage across the gill and other external surface epithelia. In invertebrates, the cellular immune system, gut epithelium, and hepatopancreas are likely to be targeted [22]. Recent studies with Mozambique tilapia (*Oreochromis mossambicus*) have indicated that nickel NPs may be internalized via these routes [23]. In addition, NPs that are taken up via ingestion through the digestive tract may accumulate in the hepatopancreas [24]. The hepatopancreas is responsible for metabolism and detoxification

[25] and is known for intracellular lysosomal digestion of food via internalization by endocytosis [22]. Nanosize particles have also been demonstrated to enter the liver of fish.

Endocytosis (<100 nm) and phagocytosis (100–100,000 nm) represent the two processes by which NPs might be absorbed into eukaryotic cells. At the cellular level, internalizations of NPs occur via endocytosis. Iron oxide NP internalization via endocytosis has been reported by Auffan et al. [26].

NPs released into the environment are affected by environmental factors such as pH, temperature, and presence of organic matter. The pH affects NP surface charge and consequently also aggregation. This has been reported by Gilbert et al. [27] who reported a pH-driven aggregation and disaggregation with larger aggregate radius at higher pH. Furthermore, Adams and Kramer [28] reported increased mobility under increased acidification. Temperature is also known to affect aggregation. Walters et al. [11] reported that forma-tion of smaller aggregates at higher temperatures suggests higher toxicity. Liu and Hurt [14] reported higher dissolution rates of AgNPs with increased temperature. NPs can be immobilized as a result of sorption or binding to particles such as organic matter. These effects have been reported. For example, Chen and Elimelech [29] reported that, in the presence of humic acid, the adsorbed humic acid on the fullerene NPs led to steric repulsion, stabilization of the NP suspension, and reduced aggregation.

5. Mechanisms of NP toxicity

Many studies have attempted to elucidate the mechanisms of NP toxicity and distinguish between their bulk counterparts. Nanomaterials differ from their bulk counterparts in several ways, including high surface/volume ratio. Other factors such as dissolution, size, shape, aggregation state, surface coatings, and solution chemistry also influence the toxicity of NPs.

The toxicity of various NMs AgNP [5–7, 30–32]), CuO NP [19, 33], TiO_2 NP [34], and Ni NP [23] has been studied in various aquatic species, such as *Daphnia magna* [32, 33], fish [6, 35], algae [36], and marine [37] and freshwater [13] crabs. Silver, carbon, and titanium NMs are among the most widely used types NMs used as additives in cosmetics and pharmaceuticals. Also, different NMs exhibit different properties had hence have different toxicity potencies. For example, Heinlaan et al. [38] compared the toxicities of three nanometal oxides: ZnO NPs, CuO NPs, and TiO_2 NPs. ZnO NPs was determined to be the most toxic; whereas Zhu et al. [39] reported CuO NP the most potent to cytotoxicity and genotoxicity.

The assessment of NP toxicity has largely been assessed *in vitro*, reporting inducing various negative effects at different levels of cellular organization. Typical end points measured include end points examined which include mortality, as well as sublethal effects such as development, growth, respiration, malformation, oxidative stress, and gene expression. Generation of reactive oxygen species (ROS) and free radicals causes oxidative stress (activation or inhibition of the antioxidant defense system), lipid peroxidation, and DNA damage. Toxicity of NPs will be discussed further in the following sections.

5.1. Oxidative stress

Oxidative stress is referred to as an imbalance between the production of reactive oxygen species (ROS) and the cells' ability to reduce ROS, which may be as a result an increased ROS production, a decrease in the cell's defense mechanisms, or a combination of both [40]. An overproduction of ROS may induce oxidative stress, resulting in cells failing to maintain normal physiological redox-regulated functions further resulting in oxidative modification of proteins to generate protein radicals [41], initiation of lipid peroxidation [42], DNA strand breaks and modification to nucleic acids [43], modulation of gene expression [44], thereby leading to cell death and genotoxic effects [45]. To minimize the effects of ROS-oxidative damage to cellular components, biological systems have developed a complex antioxidant system, comprised of both enzymatic and non-enzymatic defense mechanisms. **Figure 4** summarizes the redox cycle including ROS generation by NPs and the antioxidant defense system.

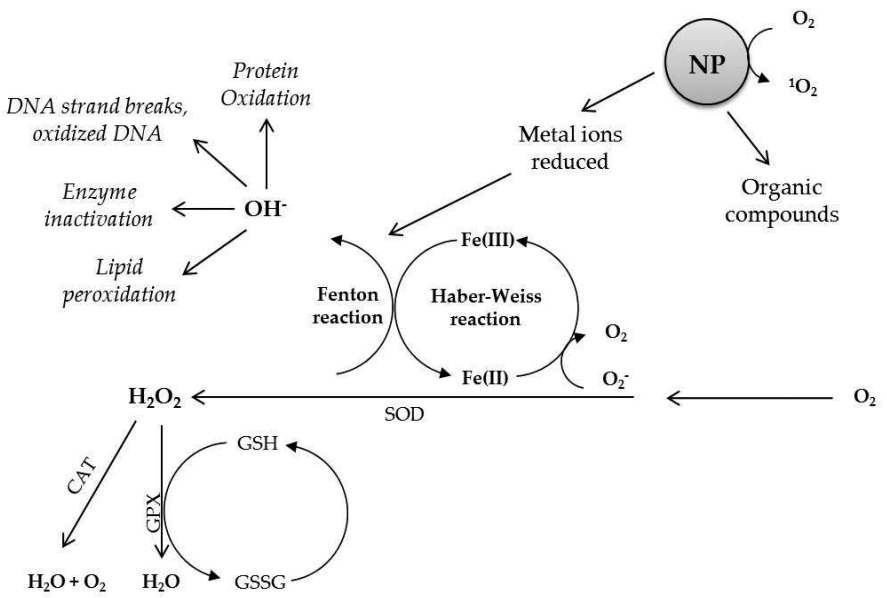

Figure 4. ROS production and defense mechanisms (adapted from Unfired et al. [46]).

The antioxidant defense system has evolved to provide a balance between the production and removal of ROS. These are catalyzed by a number of different enzymes including Phase I and Phase II enzymes. Phase I enzymes, such as cytochrome P450, initiate the detoxification process by introducing a polar moiety which renders a lipophilic contaminant more hydrophilic. Activity of Phase I enzymes typically leads to an increase in ROS production. Phase II enzymes are involved in conjugating metabolized xenobiotics to endogenous molecules. Phase III involves further modification and excretion.

The potential role of oxidative stress as a mechanism of toxicity of AgNPs was evaluated by several authors. Walters et al. [13] studied oxidative stress, viz. antioxidant enzyme activity following a 7-day exposure to AgNPs (100 nm) at 10 and 100 μg/mL. The levels of ROS and oxidative stress were concentration-dependant, with an x-fold increase compared to control levels. Federici et al. [47] measured ROS generation following 14 days of exposure to TiO_2 NPs (average particle size = 21 nm at 0.1, 0.5, or 1.0 mg/mL). The level of ROS (i.e., thiobarbituric acid-reactive substances (TBARS)) in the gills, intestine, and brain of the rainbow trout (*Oncorhynchus mykiss*) increased in a concentration-dependent manner. Statistically significant increases (twofold or more) in the gills, intestine, and brain vs. the control group were also reported. In line with these findings, Oberholster et al. [48] investigated the generation of superoxide ($O_2^{-}\cdot$) and consequent stimulation of antioxidant defense mechanisms following a 10-day exposure to spiked sediment with various concentrations of NMs. These authors also reported concentration-dependant increases in enzyme activities. In another study with AgNPs, Gomes et al. [49] reported oxidative damage for higher doses (0, 100, 300, 600, 1000, 1500 mg/kg) and exposure times (4 days vs. 28 days) in *Eisenia fetida*. Induction of oxidative stress and antioxidant enzymes by titanium oxide NP (TiO_2 NP) in *Daphnia magna* was studied by Kim et al. [34]. The authors' results indicated that the levels in activities of catalase (CAT), glutathione peroxidase (GPX), and glutathione S-transferase (GST) increase with increasing TiO_2 NP concentration. The levels of activities of superoxide dismutase (SOD) were reduced in the tissues with increasing TiO_2 NP concentration. In another study, Tedesco et al. [50] found signs of oxidative stress in the form of lysosomal instability. In line with these findings, Moore [4] reported decreased lysosomal stability resulting from apoptosis induction by AgNP.

5.2. Ecotoxicity

The potential ecotoxicity of NPs has currently provoked public and scientific dialogues due to debates around the risks and benefits of these materials. As such, studies on the ecotoxicological fate and effects of NMs have increased in recent years. There has been extensive research investigating the toxicity of NPs to aquatic organisms with several recent reviews reporting on ecotoxicology of NPs [3, 4]. Data on the biological effects of NPs indicate that NPs can be toxic to bacteria, algae, invertebrates, fish, and mammals. Nonetheless, nano-ecotoxicology studies remain poorly and unevenly distributed as most research undertaken has largely been restricted to a narrow range of test species. Most of the current ecotoxicological data pertaining to NMs have been done on *Daphnia magna*. These crustaceans represent the food and energy link between algae and fish [51]; therefore, these studies are particularly relevant. Park and Choi [32] studied the ecotoxicity effects of AgNPs to *D. magna* and reported increased mortality. Asghari et al. reported abnormal swimming in *D. magna* following exposure to AgNPs, while Heinlaan et al. [33] reported ultrastructural changes in the midgut of *D. magna* upon exposure to CuO NPs.

Nanoparticles are able to penetrate the semipermeable membranes of some aquatic organisms, thereby forming aggregates around the exoskeleton of aquatic organisms [52]. Uptake of various NPs by aquatic organisms crustacean *Daphnia magna* [33, 53], the polychaete *Nereis diversicolor* [30], and the freshwater algae Ochromonas *danica* [31] has been reported (**Figure 5**).

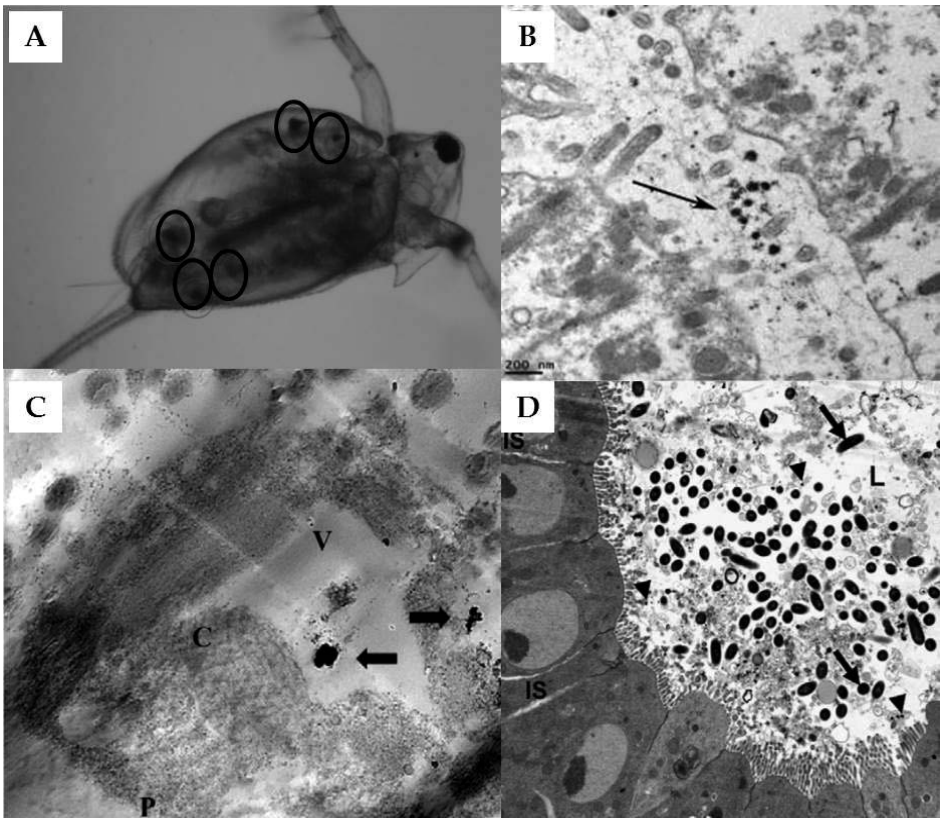

Figure 5. Nanoparticle internalization in some aquatic organisms.

Uptake of NPs generally occurs across the gills and other epithelial surfaces [6]. Scown et al. [6] reported size-dependant uptake of AgNPs (10–35 nm) and associated oxidative stress in the gills of *Danio rerio,* while Maria et al. [54] reported reduced LPO levels in gills and hepatopancreas of female *Carcinus maenas*. In crustaceans, toxicants are largely sequestered in the hepatopancreas and gills [55]. Walters et al. [13] reported higher levels on enzymatic activities in the hepatopancreas when compared to the gills suggesting that the hepatopancreas might be a more sensitive organ [56] to AgNP exposure and also implies a lower ability to scavenge O_2^- [37].

Studies showing enhanced ecotoxicity of NPs when compared to their bulk counterparts have led to the assumption that NPs generally represent a more potent threat. Once such study investigated the toxicity of various NPs with bulk counterparts [57], the authors reported significantly differences in toxicity (24-h LC_{50}) between Al_2O_3 NPs (82 mg/L) and bulk Al_2O_3 (153 mg/L) and between TiO_2 NPs (80 mg/L) and bulk TiO_2(136 mg/L).

Studies reporting on reproduction and developmental end points are common. Wu et al. [58] recently showed that AgNPs induced a variety of morphological malformations such as edema, spinal abnormalities, fin fold abnormalities, heart malformations, and eye defects in Japanese

medaka (*Oryzias latipes*). Wiench et al. [59] performed a 21-day chronic Daphnia reproduction study using coated TiO_2 NPs and reported that the NOEC for adult mortality was 30 mg/L, while the NOEC for offspring production was 3 mg/L. The 21-day EC10 and EC50 values for reproductive effects were 5 mg/L and 26.6 mg/L, respectively.

5.3. Genotoxicity

An important issue relating to the toxicity of NPs in biological media is the ability to cause damage to the genetic material, particularly since NPs have the capacity to cross cell membranes. In the section below, evidence of NP-induced genotoxicity is reviewed. DNA is a significant cellular component highly susceptible to oxidative damage. As such, there has been increasing interest in the analysis of the potential nanoparticle genotoxicity to aquatic organisms.

Genotoxic assessments of various NPs have largely been reported on in *vitro* studies. Reported abilities of NPs include chromosomal fragmentation, DNA strand breakages, point mutations, oxidative DNA adducts, and alterations in gene expression profiles and consequently may initiate and promote mutagenesis and carcinogenesis. Primary genotoxicity stemming from the direct interactions of NP with DNA following NP internalization has been reported [60]. Genotoxicity mediated by the generation of excess ROS, referred to as secondary genotoxicity, has been reported. Oberholster et al. [48], using DNA strand breakage as an indicator of genotoxicity, reported concentration-dependent effects to several NPs (α-alumina, β-alumina, precipitated silica; silica fume, calcined silica fume, colloidal antimony pentoxide, and superfine amorphous ferric oxide). DNA cleavage, an indicator of irreversible completion of apoptosis, occurred in organisms exposed to 5000 μg/kg of precipitated silica, amorphous ferric oxide, and colloidal antimony pentoxide NMs. The inter-nucleosomal DNA ladderbands occurred at 500 μg/kg of γ-alumina and α-alumina.

As with NP toxicity, NPs are also known to have more adverse genotoxic effects than their bulk counterparts. For example, Park and Choi [32] studied the genotoxicity of AgNPs on the freshwater crustacean *Daphnia magna*. Their results reported a higher degree of DNA damage in the form of DNA strand breaks in AgNPs when compared to Ag ions. Similarly, NP size is also known to affect its genotoxicity potential, inducing significant DNA and chromosomal damages compared to the larger NPs. This size effect was confirmed: the authors showed that smaller sized TiO_2 NPs (10 nm) have significant chromosomal damage when compared to the larger TiO_2NP (>200 nm) [61].

As such, there is a general consensus that smaller sized NPs produce higher reactivity and thus higher genotoxicity [62, 63]. However, particle size is not the only factor that determines particle (geno-)toxicity. Nanoparticle surface coating has also been reported to promote genotoxicity. Surface coating modifies the particle surface, and therefore, they may also alter the particle's genotoxicity. For instance, Hong et al. [64] reported positively charged coatings of iron oxide NPs which consequently resulted in increased DNA strand breaks, while the impact of genotoxicity of negatively charged coatings was insignificant. Similarly, Lui et al. [65] reported various genotoxic responses of iron oxide NPs depending on the type of coating.

polyethylene glycol (PEG) coating exhibited mutagenic activity, while solid electrolyte interphase (SEI) exhibited no genotoxicity.

As with ecotoxicological effects, genotoxicity effects of coated NPs have also been investigated. Ahamed et al. [66] reported that coated AgNPs resulted in more upregulation of these proteins, suggesting that coated Ag NPs causes greater genotoxicity than uncoated AgNP. In similar study, AshaRani et al. [35] investigated the genotoxicity of coated silver NPs vs. uncoated silver NPs in the zebrafish embryos (*Danio rerio*). The authors reported that NPs which penetrated the nucleus cells able to take alterations and breakings to DNA.

6. Conclusions

The use of NMs in consumer products and their potential environmental and human health risks is of increasing concern. As nanotechnologies and products increase, nanoproducts entering the aquatic ecosystems and other water sources too will increase, thereby increasing the potential threat to aquatic organisms. This chapter provides a review of nanotoxicology—an emerging multidisciplinary field of science—with special focus on the effects of metal-NMs on aquatic invertebrates. NMs, depending on the size, shape, elemental materials, and the surface functional groups, induce oxidative stress thus leading to (nano)toxicity and genotoxicity. The risks associated with NMs (i.e., its fate, behavior, and toxicity in the environment) are largely unknown and difficult to predict. As the ultimate sink for conventional contaminants, the aquatic ecosystem is therefore predisposed to the potential effects of NPs.

Although our knowledge on the toxicity of various NMs in the aquatic environment has increased over the past few years, there is still a lack of knowledge regarding exposure concentrations, bioaccumulation in tissues, as well as environmental factors which could potentially affect its toxicity or bioaccumulation. Exposure to NPs is inevitable since NPs become more widely used, but there remains much more to be understood regarding their safety.

Although current toxicity testing protocols is generally applicable to identify deleterious effects associated with NPs, the mechanisms of action that govern toxicity of NMs are the subject of ongoing research. Research into new analytical methods is also required to address the special properties of NMs. The outcomes will thus enable researchers to predict the toxicological effects of AgNPs with the intent of guiding its development, application, and regulation. This will be important when considering measures for exposure control and environmental remediation of AgNPs.

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References

[1] Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochelle Jr MF, Rejeski D, Hull MS. Nanotechnology in the real world: redeveloping the nanomaterials consumer products inventory. Journal of Nanotechnology. 2015;6:1769–1780. doi:10.3762/bjnano.6.181.

[2] Toensmeier PA. Nanotechnology faces scrutiny over environment and toxicity. Plastics Engineering. 2004;60(11):14–17.

[3] Oberdorster E, Zhu SQ, Blickley TM, Clellan-Green P, Haasch ML. Ecotoxicology of carbon-based engineered nanoparticles: effects of fullerene (C-60) on aquatic organisms. Carbon. 2006;44(6):1112–1120. doi:10.1016/j.carbon.2005.11.008.

[4] Moore MN. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environment International. 2006;32(8):967–976. doi:10.1016/j.envint.2006.06.014.

[5] Lankvel DPK, Oomen AG, Krystek P, Neigh A, Troost-de Jong A, Noorlander JCH, Geertsma RE, De Jong WH. The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomaterials. 2010;31:8350–8361. doi:10.1016/j.biomaterials.2010.07.045.

[6] Scown TM, Santaos EM, Johnston BD, GAiser B, Baalousha M, Mitov S, Lead JR, Stone V, Fernandes TF, Jepson, M, van Aerle R, Tyler CR. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. Toxicological Sciences. 2010;115(2):521–534. doi:10.1093/toxsci/kfq076.

[7] Gaiser BK, Biswas A, Rosenkranz P, Jepson MA, Lead JR, Stone V, Tyler CR, Fernandes TF. Effects of silver and cerium dioxide micro- and nano-sized particles on Daphnia magna. Journal of Environmental Monitoring. 2011;13:1227–1235. doi:10.1039/c1em10060b.

[8] Monrones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Tacaman MJ. The Bacterial effect of silver nanoparticles. Nanotechnology. 2005;16(10):2346–2353. doi:10.1088/0957-4484/16/10/059.

[9] Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacteria Escherichia coli. Applied Environmental Microbiology. 2007;73(6):1712–1720. doi:10.1128/AEM.02218-06.

[10] Romer I, White TA, Baalousha M, Chipman K, Viant MR, Lead JR. Aggregation and dispersion of silver nanoparticles in exposure media for aquatic toxicity tests. Journal of Chromatography, A. 2011;1218(27):4226–4233. doi:10.1016/j.chroma.2001.03.034.

[11] Walters C, Pool E, Somerset V. Aggregation and dissolution of silver nanoparticles in a laboratory-based freshwater microcosm under simulated environmental conditions. Toxicology and Environmental Chemistry. 2013;95(10):1690–1701. doi: 10.1080/02772248.2014.904141.

[12] Zhang W, Yao Y, Li K, Huang Y, Chen Y. Influence of dissolved oxygen on aggregation kinetics of citrate-coated silver nanoparticles. Environmental Pollution. 2011;159:3757–3762. doi:10.1016/j.envpol.2011.07.013.

[13] Walters CR, Cheng P, Pool E, Somerset V. Effect of temperature on oxidative stress parameters and enzyme activity in the tissues of cape river crab (Potamonautes perlatus) following exposure to silver nanoparticles (AgNP). Journal of Toxicology and Environmental Health, Part A. 2016;79(2):1–11. doi:10.1080/15287394.2015.1106357.

[14] Liu J, Hurt RH. Ion Release kinetics and particle persistence in aqueous nano-silver colloids. Environmental Science and Technology. 2010;44(6):2169–2175. doi:10.1021/es9035557.

[15] Powers KW, Brown SC, Krishna VB, Wasdo SC, Moudgil BM, Roberts SM. Research strategies for safety evaluation of nanomaterials, part IV: characterization of nanoscale particles for toxicological. Toxicology Science. 2006;90(2):296–303. doi: 10.1093/toxsci/kfj099

[16] Baalousha M. Aggregation and disaggregation of iron oxide nanoparticles: influence of particle concentration, pH, and natural organic matter. Science of the Total Environment. 2009;407:2093–2101. doi:10.1016/j.scitotenv.2008.11.022.

[17] El Badawy AM, Silva RG, Morris B, Scheckel KG, Suidan MT, Tolaymat TM. Surface charge-dependent toxicity of silver nanoparticles. Environmental Science and Technology. 2011;45(1):283–287. doi:10.1021/es1034188.

[18] Murdock RC, Braydich-Stolle L, Schrand AM, Schlager JJ, Hussain SM. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicological Sciences. 2008;101(2):239–253. doi:10.1093/toxsci/kfm240.

[19] Perez S, Farre M, Barcelo D. Analysis, behavior and ecotoxicity of carbon-based nanomaterials in the aquatic environment. Trends in Analytical Chemistry. 2009;28:820–832. doi:10.1016/j.trac.2009.04.001.

[20] Kishore PS, Viswananthan B, Varadarajan TK. Synthesis and characterization of metal nanoparticles embedded conducting polymer-polyoxometalate. Nanoscale Research Letters. 2008;3:14–20. doi:10.1007/s11671-007-9107-z.

[21] Klaine SJ, Alvarez PJJ, Batley GE, Gernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR. Nanomaterials in the environment: behaviour, fate, bioavailability, and effects. Environmental Toxicology and Chemistry. 2008;27(9):1825–1851. doi:10.1897/08-090.1.

[22] Moore NM. Lysosomal cytochemistry in marine environmental monitoring. Histochemistry. 1990;22:187–191. doi:10.1007/BF02386003.

[23] Jayaseelan C, Rahuman AA, Ramkuma R, Perumal P, Rajakumar G, Kirthi G, Santhoshkumar AV, Marimuthu S. Effects of sub-acute exposure to nickel nanoparticles on oxidative stress and histopathological changes in Mozambique tilapia Oreochromis mossambicus. Ecotoxicology and Environmental Safety. 2014;107:220–228. doi:10.1016/j.ecoenv.2014.06.012.

[24] Lee RF. Bioavailability, biotransformation and fate of organic contaminants in estuarine animals. In: Newman MC, Roberts MH Jr, Hale RC, editors. Coastal and Estuarine Risk Assessment. CRC Press; 2001. p. 97–126. doi:10.1201/9781420032451.ch5.

[25] Saravana Bhavan P, Geraldine P. Biochemical stress responses in tissues of the prawn Macrobrachium malcolmsonii on exposure to endosulfan. Pesticide Biochemistry and Physiology. 2001;70(1):27–41. doi:10.1006/pest.2001.2531.

[26] Auffan M, Decome L, Rose J, Orsiere T, DeMeo M, Briois V, Chaneac C, Olivi L, Berge-Lefranc JL, Botta A, Wiesner MR, Bottero JY. In vitro interactions between DMSA-coated maghemite nanoparticles and human fibroblasts: a physiological and cyto-genotoxical study. Environmental Science and Technology. 2006;40(14):4367–4373. doi:10.1021/es060691k.

[27] Gilbert B, Lu G, Kim CS. Stable cluster formation in aqueous suspensions of iron oxyhydroxide nanoparticles. Journal of Colloid and Interface Science. 2007;313(1):152–159. doi:10.1016/j.jcis.2007.04.038.

[28] Adams NWH, Kramer JR. Silver speciation in wastewater effluent, surface waters, and pore waters. Environmental Toxicology and Chemistry. 1999;18:2667–2673. doi:10.1897/1551-5028(1999)018<2667:SSIWES>2.3.CO;2.

[29] Chen KL and Elimelech M. Influence of humic acid on the aggregation kinetics of fullerene (C60) nanoparticles in monovalent and divalent electrolyte solutions. Journal of Colloid and Interface Science. 2007;309:126–134. doi:10.1016/j.jcis.2007.01.074.

[30] Garcia-Alonso J, Khan FR, Misra SK, Turmaine M, Smith BD, Rainbow PS, Luoma SN, Valsami-Jones E. Cellular internalization of silver nanoparticles in gut epithelia of the estuarine polychaete Nereis diversicolor. Environmental Science and Technology. 2011;45:4630–4636. doi:10.1021/es2005122.

[31] Miao A-J, Luo Z, Chen C-S, Chin W-S, Santschi PH, Quigg A. Intracellular uptake: a possible mechanisms for silver engineered nanoparticle toxicity to a freshwater alga Ochromonas danica. PLoS One. 2010;5(12):e15196. doi:10.1371/journal.pone.0015196.

[32] Park S-Y and Choi J. Geno- and ecotoxicity evaluation of silver nanoparticles in freshwater crustacean Daphnia magna. Environmental Engineering Research. 2010;15(1):23–27. doi:10.4491/eer.2010.15.1.428.

[33] Heinlaan M, Kahru A, Kasemets K, Arbeille B, Prensier G, Dubourguier H-C. Changes in the Daphnia magna midgut upon ingestion of copper oxide nanoparticles: a transmission electron microscopy study. Water Research. 2011;45:179–190. doi:10.1016/j.watres.2010.08.026.

[34] Kim KT, Klaine SJ, Cho J, Kim S-H and Kim SD. Oxidative stress responses of Daphnia magna exposed to TiO_2 nanoparticles according to size fraction. Science of the Total Environment. 2010;408(10):2268–2272. doi:10.1016/j.scitotenv.2010.01.041.

[35] AshaRani, PV, Wu YL, Gong Z, Valiyaveettil S. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology. 2008;19(25):279–290. doi: 10.1088/0957-4484/19/255102.

[36] Oukarroum A, Bras S, Perreault F, Popovic R. Inhibitory effects of silver nanoparticles in two green algae, Chlorella vulgaris and Dunaliella tertiolecta. Ecotoxicology and Environmental Safety. 2012;78(1):80–85. doi:10.1016/j.ecoenv.2011.11.012.

[37] Pan L, Zhang H. Metallothionein, antioxidant enzymes and DNA strand breaks as biomarkers of Cd exposure in a marine crab, Charybdis japonica. Comparative Biochemistry and Physiology, Part C. 2006;144(1):67–75. doi:10.1016/j.cbpc.2006.06.001.

[38] Heinlaan M, Ivask A, Blinova I, Dubourguier H-C, Kahru A. Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. Chemosphere. 2008;71(7):1308–1316. doi:10.1016/j.chemosphere.2007.11.047.

[39] Zhu X, Hondroulis E, Liu W, Li C-Z. Biosensing approaches for rapid genotoxicity and cytotoxicity assays upon nanomaterial exposure. Small. 2013;9(9–10):1821–1830. doi: 10.1002/smll.201201593.

[40] Walters C, Pool E, Somerset V. Nanotoxicity in aquatic invertebrates. In: Larramendy ML, Soloneski S, editors. Invertebrates—Experimental Models in Toxicity Screening. 1st ed. Croatia: InTech; 2016. p. 13–34. doi:10.5772/61715. Available from: http://www.intechopen.com/books/invertebrates-experimental-models-in-toxicity-screening/nanotoxicity-in-aquatic-invertebrates

[41] Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. 1997. Chemical Research and Toxicology; 10(5):485–494. doi:10.1021/tx960133r.

[42] Butterfield DA, Kanski J. Brain protein oxidation in agerelated neurodegenerative disorders that are associated with aggregate proteins. Mechanisms of Ageing and Development. 2011;122(9):945–962. doi:10.1016/S0047-6374(01)00249-4.

[43] Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. Mutation Research. 2004;567(1):1–61. doi:10.1016/j.mrrev.2003.11.001.

[44] Shi H, Hudson LG, Liu KJ. Oxidative stress and apoptosis in metal ion-induced carcinogenesis. Free Radical Biology and Medicine. 2004;37(5):582–593. doi:10.1016/j.freeradbiomed.2004.03.012.

[45] Xia Q, Chiang H-M, Zhou Y-T, Yin J-J, Liu F, Wang C, Guo L, Fu PP. Phototoxicity of kava—formation of reactive oxygen species leading to lipid peroxidation and DNA damage. The American Journal of Chinese Medicine. 2012;40(6):1271–1288. doi:10.1142/S0192415X12500942.

[46] Unfired K, Albrecht C, Klotz L-O, Von Mikecz A, Grether-Beck S, Shins RPF. Cellular responses to nanoparticles: target structures and mechanisms. Toxicology. 2007;1(1):52–71. doi:10.1080/00222930701314932.

[47] Federici G, Shaw BJ, Hand RD. Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchus mykiss): gill injury, oxidative stress, and other physiological effects. Aquatic Toxicology. 2007;84(4):415–430. doi:10.1016/j.aquatox.2007.07.009.

[48] Oberholster PJ, Musee N, Noth A-M, Chelule PK, Focke WW, Ashton PJ. Assessment of the effect of nanomaterials on sediment-dwelling invertebrate Chironomus tentans larvae. Ecotoxicology and Environmental Safety. 2011;74:416–423. doi:10.1016/j.ecoenv.2010.12.012.

[49] Gomes SIL, Hansen D, Scott-Fordsman JJ, Amorim MJB. Effects of silver nanoparticles to soil invertebrates: oxidative stress biomarkers in Eisenia fetida. Environmental Pollution. 2015;199:49–55. doi:10.1016/j.envpol.2015.01.012.

[50] Tedesco S, Doyle H, Blasco J, Redmond G, Sheehan D. Exposure of the blue mussel, Mytilus edulis, to gold nanoparticles and the pro-oxidant menadione. Comparative Biochemistry and Physiology, Part C. 2010;151(2):167–174. doi:10.1016/j.cbpc.2009.10.002.

[51] Farre M, Gajda-Schrantz K, Kantiani L, Barcelo D. Ecotoxicology and analysis of nanomaterials in the aquatic. Analytical and Bioanalytical Chemistry. 2009;393(1):81–95. doi:10.1007/s00216-008-2458-1.

[52] Baun A, Sorensen SN, Rasmussen RF, Hartmann NB, Koch CB. Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates. Aquatic Toxicology. 2008;86(13):379–387. doi:10.1016/j.aquatox.2007.11.019.

[53] Asghari S, Johari SA, Lee JH, Kim YS, Jeon YB, Choi HJ, Moon MC, Yu IJ. Toxicity of various silver nanoparticles compared to silver ions on Daphnia magna. Journal of Nanobiotechnology. 2012;10(14):14–34. doi:10.1186/1477-3155-10-14.

[54] Maria VL, Santos MA, Bebianno MJ. Contaminants effects in shore crabs (Carcinus maenas) from Ria Formosa Lagoon. Comparative Biochemistry and Physiology, Part C. 2009;150(2):196–208. doi:10.1016/j.cbpc.2009.04.013.

[55] Barka S. Insoluble detoxification of trace metals in a marine copepod Tigriopus brevicornis (Muller) exposed to copper, zinc, nickel, cadmium, silver and mercury. Ecotoxicology. 2007;16(7):491–502. doi:10.1007/s10646-007-0155-z.

[56] Hao L, Wang Z, Xing B. Effect of sub-acute exposure to TiO_2 nanoparticles on oxidative stress and histopathological changes in juvenile carp (Cyprinus carpio). Journal of Environmental Sciences. 2009;21(10):1459–1466. doi:10.1016/S1001-0742(08)62440-7.

[57] Wang H, Wick RL, Xing B. Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode Caenorhabditis elegans. Environmental Pollution. 2009;157:1171–1177. doi:10.1016/j.envpol.2008.11.004.

[58] Wu Y, Zhou Q, Li H, Liu W, Wang T, Jiang G. Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (Oryzias latipes) using the partial-life test. Aquatic Toxicology. 2010;100(2):160–167. doi:10.1016/j.aquatox.2009.11.014.

[59] Wiench K, Wohlleben W, Hisgen V, Radke K, Salinas E, Zok S, Landsiedel R. Acute and chronic effects of nano- and non nano-scale TiO_2 and ZnO particles on mobility and reproduction of the freshwater invertebrate Daphnia magna. Chemosphere. 2009;76(10):1356–1365. doi:10.1016/j.chemosphere.2009.06.025.

[60] Landsiedel R, Kapp MD, Schulz, M, Wiench K, Oesch F. Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations – many questions, some answers. Mutation Research. 2009;681(2–3):241–258. doi:10.1016/j.mrrev.2008.10.002.

[61] Gurr JR, Wang AS, Chen CH, Jan KY. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology. 2005;213:66–73. doi:10.1016/j.tox.2005.05.007.

[62] Ordzhonikidze CG, Ramaiyya LK, Eqorova EM, Rubanovich AV. Genotoxic effects of silver nanoparticles on mice in vivo. Acta Naturae (Russia). 2009;1(3):99–101.

[63] Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, Park JD, Choi BS, Lim R, Chang HK Chung YH, Kwon IH, Han BS, Yu IJ. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhalation Toxicology. 2008;20(6):575–583. doi:10.1080/08958370701874663.

[64] Hong SC, Lee JH, Lee J, Kim HY, Park JY, Cho J, Han DW. Subtle cytotoxicity and genotoxicity differences in the superparamagnetic iron oxide nanoparticles coated with

various functional groups. International Journal of Nanomedicine. 2011;6:3219–3231. doi:10.2147/IJN.S26355.

[65] Lui Y, Xia Q, Liu Y, Zhang S, Cheng F, Zhong Z, Wang L, Li H, Xiao K. Genotoxicity assessment of magnetic iron oxide nanoparticles with different particle sizes and surface coatings. Nanotechnology. 2014;25(42):425101. doi: 10.1088/0957-4484/25/42/425101.

[66] Ahamed M, Karns M, Goodson M, Rowe J, Hussain SM, Schlager JJ, Hong Y. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicology and Applied Pharmacology. 2008;233(3):404–410. doi:10.1016/j.taap.2008.09.015.

Chapter 4

Potential Harm of Maltodextrin-Coated Cadmium Sulfide Quantum Dots in Embryos and Fetuses

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Additional information is available at the end of the chapter

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Abstract

Over the past years, there has been significant interest in the study of nanoparticles for clinical applications, particularly quantum dots (QDs). However, previous studies have also shown that QDs can reach the embryo through the placenta, a natural barrier for a large variety of organic substances with diverse molecular structures, and may cause developmental deformities. Due to its essential role in a toxicological profile and its relevance to human safety, knowledge regarding embryotoxicity is of great importance. Previous studies by this research group have shown that CdS-maltodextrin QDs are biocompatible and nontoxic to cells and animals; however, QDs are able to induce embryotoxic effects. Therefore, as an effort to further address the issue, we studied the effects of CdS-maltodextrin QDs on embryo and fetus development using an embryotoxicity and teratogenicity assay on chicken embryos. Chicken embryos exposed to CdS-maltodextrin QDs (0.001, 0.01, 0.1 and 1 μg/kg) *in ovo* for 72 h showed growth and developmental alterations during the early stage and at the end of their development in a dose-dependent manner. Decreased development was observed during early stages (Stages 9/10 on the Hamburger-Hamilton scale) when compared with untreated eggs (Stage 13). Chicken embryos exposed to lower CdS-maltodextrin QDs doses (0.01, 0.1 and 1 ng/kg) and incubated *in ovo* for 21 h also showed growth and development alterations during the early stages and at the end of their development in a dose-dependent manner. However, reduced development was observed at the end of the development period (21 days), and this was associated with death of the chick. Current studies have also shown that CdS-dextrin induces embryotoxicity and teratogenicity, affecting mainly the CNS, the neural tube and somites in chicken embryos. The nature of the observed abnormalities suggests that these effects could be directly associated with nanoparticle concentrations affecting somitogenesis. Therefore, according to the

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results, there is a high probability that the prolonged accumulation of QDs in the maternal organism may be potentially harmful on embryo and fetus development. This study is limited to the analysis of embryotoxic and teratogenic effects induced by CdS-maltodextrin QDs.

Keywords: embryotoxicity, teratogenicity, nanoparticles, quantum dots, embryos

1. Introduction

Countless publications about of the *in vitro* and *in vivo* effects of nanomaterials have appeared over the past years [1]. It is now well known that the same properties that make nanoparticles so attractive to biomedicine may contribute to their toxicological profile in biological systems [2, 3]. Nevertheless, there are still many unanswered questions about their toxicological aspects, such as embryotoxicity and teratogenicity.

It is well known that embryos and fetuses are most sensitive to harmful factors during critical periods [4]. Not only is differentiation during organogenesis a highly susceptible period to the induction of malformations, but also the fetal/neonatal developmental phases are just as prone to certain developmental deficits [5]. Therefore, embryotoxicity and teratogenicity assays are of great importance given their indispensable role in the toxicological profile that must be established for any new active substance relevant to human safety, including nanomaterials. In recent years, different studies have explored the passage of nanomaterials through the placenta, a natural barrier for a large variety of organic substances with diverse molecular structures [6–8]. It is now known that the passage of nanomaterials through the placental barrier may affect fetal cell proliferation, embryonic growth, and organ formation [9]. Depending on the doses and time of exposure—from fertilization through the fetal period and eventually during lactation—the consequences can range from embryotoxicity to gross malformations, and a large variety of more subtle morphological, biochemical, and functional abnormalities have been detected [10–12].

However, knowledge concerning nanomaterial embryotoxicity and teratogenicity is yet limited because the toxicity of each nanoparticle depends on size, shape, and even surface cover, and so different nanomaterials may yield contradictory effects [13]. Because of this, in-depth knowledge of nanotoxicity and increased efforts devoted to the study of the toxic effects of nanomaterials on embryos and fetuses should be considered mandatory, as is with other investigational new drugs.

Our group is interested in the biomedical application of maltodextrin-coated cadmium sulfide QDs, semiconductor nanoparticles of about 3.5 nm in size with superior optical properties when compared to conventional organic dyes [14]. Although the *in vitro* studies revealed that these CdS-maltodextrin QDs produced distinct dose-dependent toxic effects, *in vivo* studies demonstrated that, when administered to rodents, CdS-maltodextrin QDs were biocompatible and nontoxic after 5 and 15 days [15]. Moreover, the pharmacokinetic data clearly showed that CdS-maltodextrin QDs were not completely cleared from *in vivo* systems after 360 h. Then,

although CdS-maltodextrin QDs appear to be nanomaterials with favorable pharmacokinetic properties to develop novel therapeutic and diagnostic modalities, according to previous results, CdS-maltodextrin QDs seem to be allowed into the body through several barriers and then pass into the blood stream, from where they can reach organs and tissues and interact with biological structures [16].

Early results by this group demonstrated that CdS-maltodextrin QDs were embryotoxic in a chicken embryo model [14]. The nature of the observed abnormalities suggests that these effects could be directly associated with nanoparticle concentrations. The observed effects indicate that prolonged accumulation of QDs in the maternal organism may increase the risk of adverse effects on embryo development. Since nanotoxicity studies targeting the reproductive and developmental aspects are rather scant, and considering that QD mechanisms of action during embryogenesis are not fully understood, this study aims at further addressing the issue. We studied the effects of CdS-maltodextrin QDs on embryo and fetus development using an embryotoxicity and teratogenicity assay on chicken embryos.

2. Materials and methods

2.1. Embryotoxicity study

Fertile White Leghorn chicken eggs were obtained from A.L.P.E. S.A. (Puebla, Mexico) and stored at 6°C. One hundred fertilized eggs were weighed, sterilized, and divided into nine groups. The first group served as a nontreated control and was considered the negative control. The second group was treated with 1 mL of Ringer solution. The third group was treated with caffeine (10 mg/mL) and was considered a positive control. The remaining six groups received CdS-maltodextrin QDs in different concentrations (0.001, 0.01, 0.1, 1 μg/kg). One of these groups received caffeine (10 mg/mL) and was considered the positive control. An embryotoxicity assay was carried out as described by Jelinek and Marthan [17]. Test solutions (1 mL) were added to the air sac under sterile conditions. Each solution was injected after drilling into the shell at the blunt end of the egg; after injection, the holes were immediately sealed with melted paraffin wax. The eggs were then transferred to and maintained in a forced draft incubator at 37.5°C with a relative humidity of 65% until the desired stage of development was reached (72 h).

Embryos in each group were fixed in buffered formal saline (pH 7.4), dehydrated, and embedded in paraffin blocks. Paraffin tissue sections of 6 μm were stained with acetocarmine for routine histological examination. The embryo was examined and staged according to morphological criteria previously outlined by Hamburger and Hamilton [18]. Malformations were considered for the following specific structures: central nervous system (CNS), lens placode, otic placode, cardiovascular system (CVS), neural tube, as well as number of somites. Embryonic stages at the time of the CdS-maltodextrin QD application varied from 14 to 16, which corresponds approximately to developed somites numbered 22–28. In order to monitor their uptake and distribution, we observed the embryos under a confocal microscope (Zeiss

LSM510, USA). CdS-Dx QDs were excited with 488 nm laser, and their signals were collected from 515 nm.

2.2. Teratogenicity assay

Fertile White Leghorn chicken eggs were obtained from A.L.P.E. S.A. (Puebla, Mexico) and stored at 6°C. One hundred fertilized eggs were weighed, sterilized, and divided into four groups. The first group served as a nontreated control and was considered the negative control. The other groups received CdS-maltodextrin QDs in different concentrations (0.001, 0.01, 0.1, 1 μg/kg). A teratogenicity assay was carried out as described by Jelinek and Marthan [17]. Test solutions (1 mL) were added to the air sac under sterile conditions. Each solution was injected after drilling into the shell at the blunt end of the egg; after injection, the holes were immediately sealed with melted paraffin wax. The eggs were then transferred to and maintained in a forced draft incubator at 37.5°C with a relative humidity of 65%. To determine the teratogenic effect of CdS-maltodextrin QDs, we allowed the chicks to fully develop until they were able to hatch by themselves). Developmental stages and the presence of malformations were measured according to the Hamburger-Hamilton scale.

2.3. Statistical analysis

Results are expressed as means + standard error of the mean (SEM). Significant differences were detected by one-way analysis of variance using GraphPad Instat V2.03 (GraphPad Software Inc., San Diego, CA). The Tukey–Kramer multiple comparisons test was used when significant variations were found. Differences were considered significant at $p < 0.05$.

3. Results

A chicken embryo model was used to assess CdS-maltodextrin QDs embryotoxicity. **Figure 1** shows a representative chicken embryo treated with different doses of CdS-maltodextrin QDs. As we can see, QD-treated chicken embryos showed developmental delays in a dose-dependent manner. Nonmalformations were found with doses of 0.001 μg/kg QDs. However, embryos treated with 0.01 μg/kg of CdS-maltodextrin QDs showed significant changes in the CNS; 38% of embryos showed malformations that included morphological alterations and anencephaly (**Figure 2**). Embryos treated with 0.1 μg/kg of CdS-maltodextrin QDs showed a significant developmental arrest at Stage 13 of the Hamburger-Hamilton scale, and 50% of embryos showed various malformations. Those embryos showed several alterations in structures including lens placodes (10%), otic placodes (10%), CNS (50%) and neural tube (29%) (**Figure 2** and **Table 1**). These embryos had a smaller number of somites than nontreated embryos. The dose that produced the most critical alterations was 1 μg/kg; it produced 50% of embryo mortality at Stage 12 of the Hamburger-Hamilton scale. Besides that, 71% of embryos had malformations, including lens placode 55%, otic placode 75%, CNS 71% (anencephaly and morphological alterations), lower number of somites, and 50% presented alterations in the neural tube (see **Figure 3** and **Table 1**).

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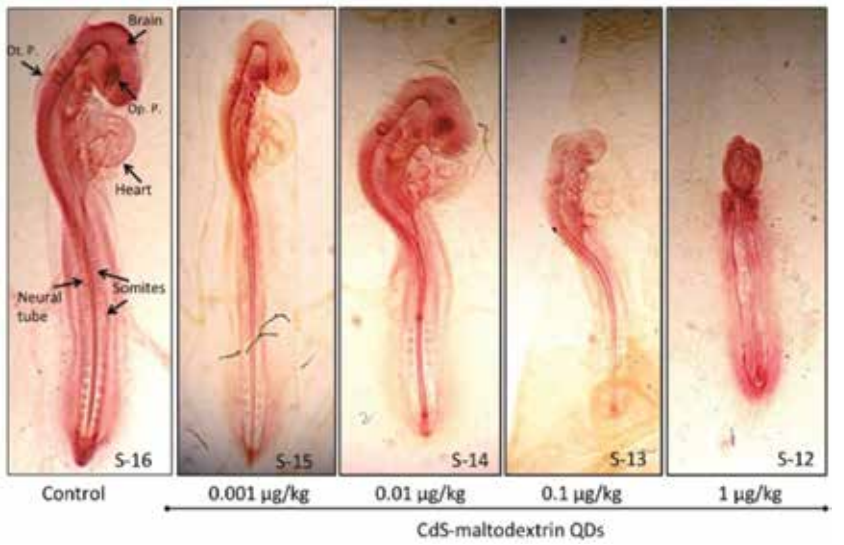

Figure 1. Effect of CdS-maltodextrin QDs on embryogenesis. Embryos were staged in accordance with the Hamburger-Hamilton scale (S), and a dose-dependent delay in embryo development was observed. Several deformities are evident. Ot. p.: otic placode; Op. p.: lens placode; S: somites; NT: neural tube (Magnification 4×).

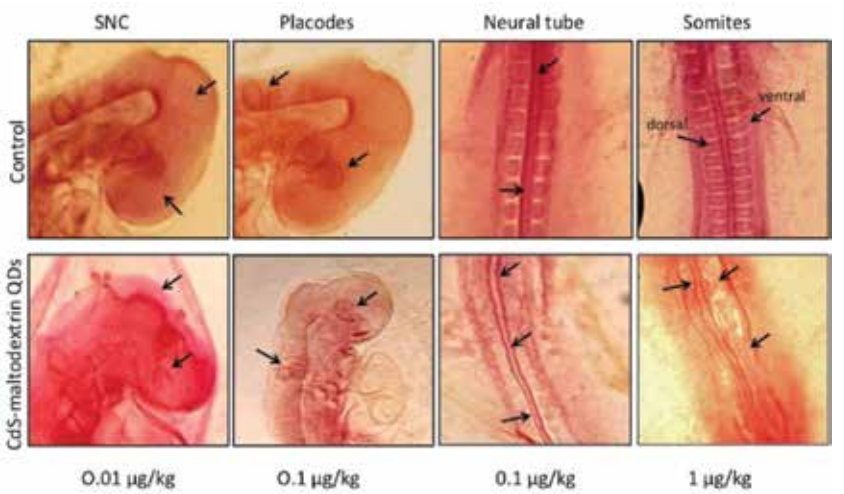

Figure 2. Gross abnormalities associated with CdS-maltodextrin QDs. Application of 0.01, 0.1 and 1 µg/kg QDs in sterile chicken ringer solution to chicken embryo *in ovo* induced overall growth delay, as well as defects in the brain, neural tube and somites, as opposed to embryos treated with sterile chicken ringer alone (arrows). Embryos treated with 0.01 µg/kg QDs showed evident brain defects. 0.1 µg/kg QDs produced brain and neural tube defects; besides those defects, 1 µg/kg QD treatments resulted in malformations. The somites formed after QD treatment lack uniform rectangular alignment and border an abnormally formed neural tube. The localization of the asterisks represents neural tube deformities. Applications of QDs caused multiple abnormalities in the otic and lens placodes. (Magnification 4×).

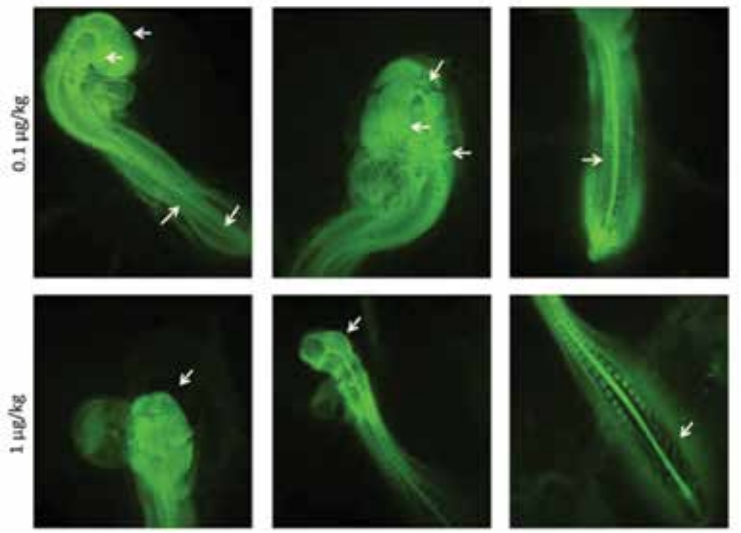

Figure 3. Fluorescence microscopic images showing the distribution and localization of CdS-maltodextrin QDs in embryos. These images correspond to embryos treated with 0.1 and 1 µg/kg. The distribution and localization of CdS-MDx QDs were identified by a bright green imaging in the analyzed embryos. There is an evident presence of abnormalities in the brain, placodes, neural tube and somites. (Magnification 4×).

Parameter	Controls			CdS-maltodextrin QDs (µg/kg)			
	Control[1]	Ringer	Caffeine	0.001	0.01	0.1	1
Viability (%)	100	100	100	100	100	100	50
Stage[2]	16 ± 0.9	16 ± 0.5	12 ± 0.8*	15 ± 1.0	14 ± 1.2	13 ± 1.1*	12 ± 2.6*
Malformations (%)	0	0	100	0	38	50	71
Type of malformations							
Optical placode (%)	0	0	100	0	0	10	55
Otic placode (%)	0	0	100	0	0	10	75
CNS (%)	0	0	100	0	100	100	100
Somites (#)	26 ± 1.1	25 ± 1.5	20 ± 2.1	25 ± 1.8	24 ± 1.9	20 ± 4.3	15± 2.1
Heart (%)	0	0	100	0	0	0	0
Neural tube (%)	0	0	100	0	13	29	50

N = 14; CNS = central nervous system.

[1]Nontreated embryos.

[2]Hamburger-Hamilton scale.

*$p < 0.05$ as compared to control group.

Table 1. Effect of CdS-maltodextrin QDs on embryos viability and development.

The fluorescent properties of CdS-maltodextrin QDs allowed us to monitor their uptake and distribution directly by observing the bright green light they emit. CdS-maltodextrin QDs uptake and bioimaging experiments were performed with a fluorescent microscope. **Figure 3** shows CdS-maltodextrin QD distribution in chicken embryos. The uniform distribution of QDs in embryonic tissues was evident. The presence of malformations on the CNS, placodes and somites with doses of 0.1 μg/kg was evident too. However, more severe malformations were witnessed with doses of 1 μg/kg, such as absence of head, brain, otic placode and somites. Somites, mainly the ventral ones, were affected in size, morphology and number in a dose-dependent manner (**Figures 2** and **3**).

The teratogenic effect of CdS-maltodextrin QDs was also evaluated using a chicken embryo model. Thus, we observed several malformations in embryos with QD doses of 0.01–1 μg/kg; for the teratogenicity study, we employed lower doses (0.01, 0.1 and 1 ng/kg) in order to allow for chick development. However, although lower doses were used as compared with the embryotoxicity study, the lack of development and birth defects was clear, and even with the same doses there were variations in embryo development. For example, embryos treated with 0.1 ng/kg QDs were at Stages 23 and 41, and embryos treated with 1 ng/kg were at Stages 20 and 32.

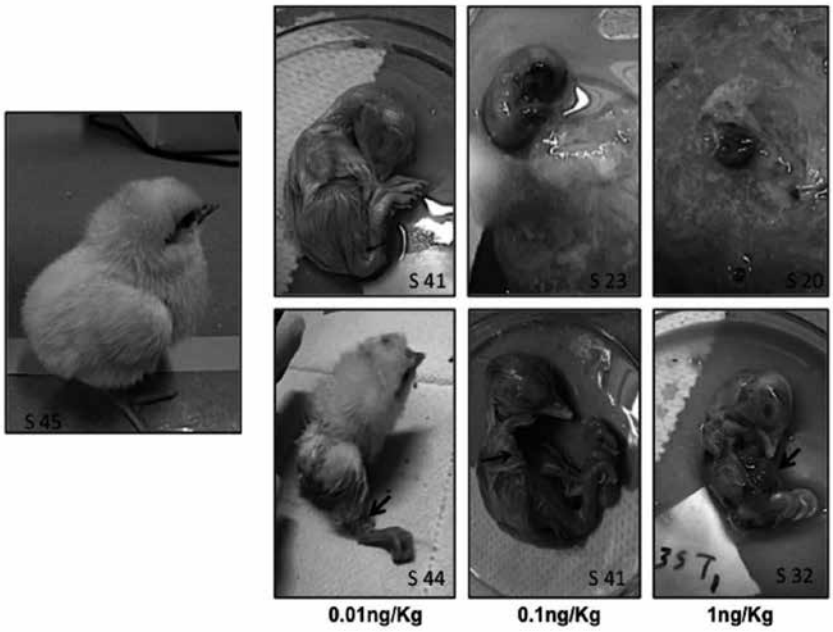

Figure 4. Morphological aspects of chicken after CdS-maltodextrin QD treatment. Chicken treated with QDs showed different deformities. Full development was only observed with the lowest dose (0.01 ng/kg). Severe malformations were observed with 0.1 and 1 ng/kg. Effects on fetus development at Stages 20 and 23 were also observed.

These results showed that the treatment of chicken embryos with CdS-maltodextrin QDs for 21 days caused reduced chicken viability (**Figure 4** and **Table 2**), and a dose-dependent

developmental delay was also observed. Only 35% of the chicks were alive and able to hatch when they were treated with 0.01 ng/kg of CdS-maltodextrin QDs. The chicks were able to hatch by themselves and did not show apparent malformations, but these chicks did not attain full development (32–36 on the Hamburger-Hamilton scale) and had a lack of lower limb motor coordination. Mortality was as high as 70% among chicks treated with 0.1 ng/kg of QDS. Of the chicks that were alive, only 20% were able to hatch by themselves, and 25% showed malformations consisting of lack of abdominal wall closure. The group of chickens treated with the highest doses (1 ng/kg) resulted in 100% mortality. These chicks showed a very early developmental stage (26–32 of the Hamburger-Hamilton scale), and it was not even possible to detect specific organs. Chicks in advanced stages presented anencephaly and a lack of abdominal wall closure (**Figure 4** and **Table 2**).

Parameters	**Controls**		**CdS-maltodextrin QDs (ng/kg)**		
	Control[1]	**Ringer**	**0.01**	**0.1**	**1**
Viability (%)	100	100	35	18	0
Hatch (%)	100	100	67	20	0
Stage (S)[2]	46	45	42 ± 3*	30 ± 7*	26 ± 6*
Malformations (%)	0	0	0	25	44.4
Type of malformations					
Malformations	None	None	Nonmalformations Lack of development Lack of motor coordination of lower limbs	Lack of closure of abdominal wall with exposed viscera	Lack of closure of abdominal wall with exposed viscera Anencephaly

N = 20.

[1]Nontreated embryos.

[2]Hamburger-Hamilton scale.

*$p < 0.05$ as compared to control group.

Table 2. Effect of CdS-maltodextrin QDs on chicken viability and development.

4. Discussion

Over the past years, there has been significant interest in the study of nanoparticles for clinical applications, particularly quantum dots (QDs). One of the most valuable QD properties is their

fluorescence spectrum, which renders them optimal fluorophores for bioimaging applications [19]. Due to their fluorescent features, QDs can be conjugated with bioactive moieties (e.g., antibodies or receptor ligands) to target specific biological events and cellular structures [20]; however, due to their small size and physical resemblance to physiological molecules, particular attention has being focused to potential risks of human beings. Understanding quantum dot potential toxicity should require a fundamental grasp of QDs properties. However, the most important problem is that each individual type of QD possesses its own unique physicochemical properties, which in turn determine its potential toxicity or lack thereof [21, 22].

Many research groups have contributed with evidence that supports the application of a precautionary approach when creating products containing nanoparticles, such as QDs [23–25]. According to their reports, there are several aspects to be considered. Firstly, QD size down to the nanoscale (between 1 and 100 nanometers); a small size allows nanoparticles to enter the body through several cellular barriers and pass into the bloodstream, from where they can reach organs and tissues and fully interact with biological structures, thus damaging normal functions in different ways [26]. Secondly, a potential source of confusion in assessing QD toxicity is that QD toxicity depends on multiple factors derived from both individual QD physicochemical properties and environmental conditions: QD charge, concentration, outer coating bioactivity (coating material, functional groups), as well as oxidative, photolytic, and mechanical stability have each been shown to be determining factors in QD toxicity [27]. The third aspect is that most nanomaterials enter the market without a toxicity analysis, and currently available testing is not suitable for a thorough assessment of its potential risks. The fourth aspect derives from several *in vitro* studies conducted on animals, which have shown that certain nanomaterials are toxic to subjects, and most likely are so to humans [28, 29]. The fifth and last aspect is that there are no regulations for nanomaterial synthesis, handling, use, and proper disposal in all countries.

We recently synthesized maltodextrin-coated cadmium sulfide QDs (CdS-maltodextrin) [14]. Although the *in vitro* studies revealed that these CdS-maltodextrin QDs produced distinct dose-dependent toxic effects, *in vivo* studies demonstrated that, when administered to rodents, CdS-maltodextrin QDs were biocompatible and nontoxic after 15 days of exposure. Nevertheless, the CdS-maltodextrin QD pattern of biodistribution and accumulation in tissue was different after repeated doses [15]. The pharmacokinetic study of those QDs clearly showed that CdS-maltodextrin QDs were not completely cleared from *in vivo* systems after 360 h after a single dose [16], suggesting that QDs may remain for long periods of time in some organs.

It is well known that biological barriers play an important role to determine QD biodistribution [30, 31]. This group has demonstrated that CdS-maltodextrin QDs are able to cross the blood–brain barrier (BBB) and spread into the brain after repeated doses in rodents without inducing morphological and functional changes [15]. However, the pharmacokinetic analysis showed that CdS-maltodextrin QDs could remain in the brain for a very long period of time. On the other hand, we have also demonstrated the presence of CdS-maltodextrin QDs in the blood–testis barrier (BTB) adjacent seminiferous tubules. The BTB was found to be intact and functional after a single dose as well as after repeated doses in rodents [15, 16]. Those results

suggest that CdS-maltodextrin QDs might also be able to cross the placental barrier, affecting embryos or fetuses. Therefore, concerned about the safety of CdS-maltodextrin QDs and as an effort to further address the issue, the present work studied the effects of CdS-maltodextrin QDs on the development of embryos and fetuses by using a chick embryo model. We used this model because it has long been appreciated that studying the embryonic chick *in ovo* provides a variety of advantages, including the potential to control the embryo's environment and its movement independently of maternal influences.

Exposure to chemicals during different stages of development—such as the preconceptional, periconceptional, embryonic, fetal and perinatal periods—has a varying impact on health. However, the embryonic period is the most critical period for embryogenesis, when mortality or different congenital anomalies are highly possible. NP direct embryotoxicity firstly depends on the ability of the compound to cross the maternal–fetal barrier, how much nanomaterial is accumulated in embryonic tissues, and its ability to induce damage [32]. Several reports have demonstrated contradictory effects of nanomaterials on embryos or their development. A decrease in embryonic weight after QD injection on the sixth day of embryogenesis (CdSe/ZnS QDs, 9.6%; CdT QDs, 6.2%) has been reported [33], whereas others have found that nanomaterials did not produce embryotoxic or teratogenic effects during embryogenesis [34]. It has been shown that NPs (nSP and TiO2s 70 and 35 nm in diameter, respectively) can cross the placental barrier in pregnant mice and cause neurotoxicity in their offspring, and then remain in the placenta, fetal liver, and fetal brain [35]. Some authors argue that some NPs (CdSe and CdTe/CdS) in different sizes, at different dosages, and with different outer capping materials can increase the rate of early-stage blastocyst death in mice and can be potentially transferred across the placenta to the fetus [32, 36]. On the other hand, some researchers have reported that the embryotoxic effects of some NPs can be modified if they are coated with silanes or by using a gold shell [37].

In the present study, we used different doses of 3.5-nm-sized CdS-maltodextrin QDs, and the dose-dependent embryotoxic effect was evident. Observation by fluorescence microscopy revealed the homogeneous distribution of QDs into the embryos. A significant concentration-related decline in embryonic growth was observed, as well as an increase in developmental defects, including various neuronal abnormalities. It has been reported that some nanomaterials induce neuronal alteration, which suggests an NP capacity to interfere with normal neurotransmission pathways [35, 38]. Recent studies have demonstrated QD-related neurotoxicity in the CNS, as well as synaptic transmission and plasticity impairments, and deteriorated brain functions in tested animals [39–41]. Another important finding from the present study was the presence of marked anomalies on somites. Somites are transient embryonic structures of the paraxial mesoderm that give rise to all off the striated muscular tissue in the adult body, the axial skeleton, and dermis during later embryogenesis. We found a significant reduction in the size of the somites n embryos treated with CdS-maltodextrin QDs, which correlates with the overall body shortening observed. When we analyzed the size of each somite separately, we observed that the somites in treated embryos tended to have a different size, morphology or absence, contrary to controls.

Birth defects result from errors during embryonic development. Normal development involves careful orchestration of multiple events, including changes in gene transcription, cell shape, cell proliferation and tissue morphogenesis. Alterations in the developmental program may have dramatic effects on the organism. Sometimes, these effects are so drastic that lethality occurs early in the development, while others may cause birth defects. We found several defects in chicken after normal periods of development: chicks with poor development; CNS alterations or even anencephaly; lack of limb motor coordination; spasticity; and a lack of abdominal wall closure. Some limited data from animal reproductive studies and nanomaterials suggest a potentially increased risk of early miscarriages, impaired growth and birth defects [42, 43]. A particular birth defect may be caused by several mechanisms, including folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption and specific receptor or enzyme-mediated teratogenesis. In addition, some drugs may be involved in multiple mechanisms leading to birth defects [44].

Somitogenesis, in particular, is a reiterated process occurring over time with strict periodicity. A pair of somites is formed every 90 min in the trunk of the chicken embryo from the anterior tip of the presomitic mesoderm (PSM). As development proceeds, ventral somitic cells migrate around the axial organs, giving rise to segmented structures such as vertebrae, intervertebral disks, and trunk and limb developments, whereas more dorsal somitic cells give rise to the dermis and all of the striated muscles of the adult body [45]. Somitogenesis can be achieved only by the integration of multiple signaling pathways involving intricate molecular machinery, cell proliferation and the genetic clock [46]. It has been reported that the use of transcription factor chemical inhibitors or genes induces severe defects in somite formation due to segmentation clock desynchronization [47]. Recent reports have shown evidence that the presence of reactive oxygen species (ROS) may affect the metabolic regulation of the ultradian biological oscillator with important pathophysiological implications for somitogenesis [48].

In our study, although higher doses were used in the embryotoxicity study, the lack of development and birth defects was clear, and even with the same doses there were variations in embryo development, which could be explained by individual susceptibility. Mortality was high, and the malformations and stages of development were not compatible with life. However, the presence of defects in limb neuromotor coordination, the lack of abdominal wall closure and neuronal alterations suggest that CdS-maltodextrin QDs might be affecting the early stages of somitogenesis. We suggest that CdS-maltodextrin QDs primarily affect the number of cells that segment together to form individual somites in chicken embryos and that this is responsible for the mortality and all the observed effects on the fetuses. It has been shown that CdS-maltodextrin QDs are able to induce cytotoxicity and cell death, alter cell proliferation and induce the production of radical oxygen species (ROS) in a dose-dependent manner [14]. QD-induced perturbations of cellular mechanisms may cause different pathophysiological processes depending on concentration and duration of exposure [49–51].

In conclusion, our data indicate that CdS-maltodextrin QDs induce embryotoxic and teratogenic effects with all doses used. QDs induced abnormalities associated with structures derived from somites in embryos and fetuses. Therefore, according to the results, there is a high probability that the prolonged accumulation of CdS-maltodextrin QDs in the maternal

organism may be potentially harmful to embryo and fetus development. However, further studies using mammalian species are needed in order to discard more toxic effects.

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References

[1] Ruenraroengsak P, Cook JM, Florence AT. Nanosystem drug targeting: facing up to complex realities. J Control Release. 2010; 141(3):265–76. doi:10.1016/j.jconrel.2009.10.032.

[2] Tang Y, Han S, Liu H, Chen X, Huang L, Li X, Zhang J. The role of surface chemistry in determining *in vivo* biodistribution and toxicity of CdSe/ZnS core-shell quantum dots. Biomaterials. 2013; 34(34):8741–55. doi:10.1016/j.biomaterials.2013.07.087.

[3] Hassan S, Singh AV. Biophysicochemical perspective of nanoparticle compatibility: a critically ignored parameter in nanomedicine. J Nanosci Nanotechnol. 2014; 14(1):402–14. doi:10.1166/jnn.2014.8747.

[4] Brendel K, Duhamel RC, Shepard TH. Embryotoxic drugs. Biol Res Pregnancy Perinatol. 1985; 6(1):1–54. doi:10.1007/s11010-011-0733-5.

[5] Thompson BL, Levitt P, Stanwood GD. Prenatal exposure to drugs: effects on brain development and implications for policy and education. Nat Rev Neurosci. 2009; 10(4):303–12. doi:10.1038/nrn2598.

[6] Barua S, Mitragotri S. Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future prospects. Nano Today. 2014; 9(2):223–43. doi:10.1016/j.nantod.2014.04.008.

[7] Chu M, Wu Q, Yang H, Yuan R, Hou S, Yang Y, Zou Y, Xu S, Xu K, Ji A, Sheng L. Transfer of quantum dots from pregnant mice to pups across the placental barrier. Small. 2010; 6(5):670–8. doi:10.1002/smll.200902049.

[8] Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, Diener PA, Zisch A, Krug HF, von Mandach U. Barrier capacity of human placenta for nanosized materials. Environ Health Perspect. 2010; 118(3):432–6. doi:10.1289/ehp.0901200.

[9] Zalgevičiene V, Kulvietis V, Bulotiene D, Didžiapetriene J, Rotomskis R. The effect of nanoparticles in rats during critical periods of pregnancy. Medicina (Kaunas). 2012; 48(5):256–64. doi:10.2147/IJN.S70092.

[10] Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, Yoshida T, Ogura T, Nabeshi H, Nagano K, Abe Y, Kamada H, Monobe Y, Imazawa T, Aoshima H, Shishido K, Kawai Y, Mayumi T, Tsunoda S, Itoh N, Yoshikawa T, Yanagihara I, Saito S, Tsutsumi Y. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol. 2011; 6(5):321–8. doi:10.1038/nnano.2011.41.

[11] Bayne K. Revised guide for the care and use of laboratory animals available. American Physiological Society. Physiologist. 1996; 39(4):199, 208–11. PMID: 8854724.

[12] Zhu X, Tian S, Cai Z. Toxicity assessment of iron oxide nanoparticles in zebrafish (Danio rerio) early life stages. PLoS One. 2012; 7(9):e46286. doi:10.1371/journal.pone.0046286.

[13] Bonfanti P, Moschini E, Saibene M, Bacchetta R, Rettighieri L, Calabri L, Colombo A, Mantecca P. Do nanoparticle physico-chemical properties and developmental exposure window influence nano ZnO embryotoxicity in Xenopus laevis? Int J Environ Res Public Health. 2015; 12(8):8828–48. doi:10.3390/ijerph120808828.

[14] Rodríguez-Fragoso P, Reyes-Esparza J, León-Buitimea A, Rodríguez-Fragoso L. Synthesis, characterization and toxicological evaluation of maltodextrin capped cadmium sulfide nanoparticles in human cell lines and chicken embryos. J Nanobiotechnol. 2012; 10(47):1–11. doi:10.1186/1477-3155-10-47.

[15] Gutiérrez-Sancha I, Reyes-Esparza J, Rodríguez-Fragoso P, García-Vázquez F, Rodríguez-Fragoso L. Bright green emitting maltodextrin coated cadmium sulfide quantum dots as contrast agents for bioimaging: a biocompatibility study. Int J Nanomed Nanosurg. 2015; 1(2). doi:10.16966/2470–3206.107.

[16] Gutiérrez-Sancha I, Reyes-Esparza J, Rodríguez-Fragoso P, Rodríguez-Fragoso L. Pharmacokinetic of maltodextrin coated cadmium sulfide quantum dots in rats. J Nanomed Biotherap Discov. 2016; 6:139. doi:10.4172/2155-983X.

[17] Jelínek R, Marhan O. Validation of the chick embryotoxicity screening test (CHEST). A comparative study. Funct Dev Morphol. 1994; 4(4):317–23. PMID: 7612953.

[18] Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. J Morphol. 1951; 88(1):49–92. PMID: 24539719.

[19] Wegner KD, Hildebrandt N. Quantum dots: bright and versatile *in vitro* and *in vivo* fluorescence imaging biosensors. Chem Soc Rev. 2015; 44(14):4792–834. doi:10.1039/c4cs00532e.

[20] Ding C, Zhu A, Tian Y. Functional surface engineering of C-dots for fluorescent biosensing and *in vivo* bioimaging. Acc Chem Res. 2014; 47(1):20–30. doi:10.1021/ar400023s.

[21] El-Ansary A, Al-Daihan S, Bacha AB, Kotb M. Toxicity of novel nanosized formulations used in medicine. Methods Mol Biol. 2013; 1028:47–74. doi:10.1007/978-1-62703-475-3_4.

[22] Landsiedel R, Fabian E, Ma-Hock L, van Ravenzwaay B, Wohlleben W, Wiench K, Oesch F. Toxico-/biokinetics of nanomaterials. Arch Toxicol. 2012; 86(7):1021–60. doi:10.1007/s00204-012-0858-7.

[23] Tang Y, Han S, Liu H, Chen X, Huang L, Li X, Zhang J. The role of surface chemistry in determining *in vivo* biodistribution and toxicity of CdSe/ZnS core-shell quantum dots. Biomaterials. 2013; 34(34):8741–55. doi:10.1016/j.biomaterials.2013.07.087.

[24] Barua S, Mitragotri S. Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future prospects. Nano Today. 2014; 9(2):223–43. doi:10.1016/j.nantod.2014.04.008.

[25] Holden PA, Nisbet RM, Lenihan HS, Miller RJ, Cherr GN, Schimel JP, Gardea-Torresdey JL. Ecological nanotoxicology: integrating nanomaterial hazard considerations across the subcellular, population, community, and ecosystems levels. Acc Chem Res. 2013; 46(3):813–22. doi:10.1021/ar300069t.

[26] Maldiney T, Richard C, Seguin J, Wattier N, Bessodes M, Scherman D. Effect of core diameter, surface coating, and PEG chain length on the biodistribution of persistent luminescence nanoparticles in mice. ACS Nano. 2011; 5(2):854–62. doi:10.1021/nn101937h.

[27] Tang Y, Han S, Liu H, Chen X, Huang L, Li X, Zhang J. The role of surface chemistry in determining *in vivo* biodistribution and toxicity of CdSe/ZnS core-shell quantum dots. Biomaterials. 2013; 34(34):8741–55. doi:10.1016/j.biomaterials.2013.07.087.

[28] Lee YK, Jeong JM, Hoigebazar L, Yang BY, Lee YS, Lee BC, Youn H, Lee DS, Chung JK, Lee MC. Nanoparticles modified by encapsulation of ligands with a long alkyl chain to affect multispecific and multimodal imaging. J Nucl Med. 2012; 53(9):1462–70. doi:10.2967/jnumed.111.092759.

[29] King-Heiden TC, Wiecinski PN, Mangham AN, Metz KM, Nesbit D, Pedersen JA, Hamers RJ, Heideman W, Peterson RE. Quantum dot nanotoxicity assessment using the zebrafish embryo. Environ Sci Technol. 2009; 43(5):1605–11. doi:10.1021/es801925c.

[30] Chu M, Wu Q, Yang H, Yuan R, Hou S, Yang Y, Zou Y, Xu S, Xu K, Ji A, Sheng L. Transfer of quantum dots from pregnant mice to pups across the placental barrier. Small. 2010; 6(5):670–8. doi:10.1002/smll.200902049.

[31] Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, Yoshida T, Ogura T, Nabeshi H, Nagano K, Abe Y, Kamada H, Monobe Y, Imazawa T, Aoshima

H, Shishido K, Kawai Y, Mayumi T, Tsunoda S, Itoh N, Yoshikawa T, Yanagihara I, Saito S, Tsutsumi Y. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol. 2011; 6(5):321–8. doi:10.1038/nnano.2011.41.

[32] Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, Diener PA, Zisch A, Krug HF, von Mandach U. Barrier capacity of human placenta for nanosized materials. Environ Health Perspect. 2010; 118(3):432–6. doi:10.1289/ehp.0901200.

[33] Zalgevičiene V, Kulvietis V, Bulotiene D, Didžiapetriene J, Rotomskis R. The effect of nanoparticles in rats during critical periods of pregnancy. Medicina (Kaunas). 2012;48(5):256–64. doi:10.2147/IJN.S70092.

[34] Agarwal R, Domowicz MS, Schwartz NB, Henry J, Medintz I, Delehanty JB, Stewart MH, Susumu K, Huston AL, Deschamps JR, Dawson PE, Palomo V, Dawson G. Delivery and tracking of quantum dot peptide bioconjugates in an intact developing avian brain. ACS Chem Neurosci. 2015; 6(3):494–504. doi:10.1021/acschemneuro.5b00022.

[35] Libralato G, Minetto D, Totaro S, Mičetić I, Pigozzo A, Sabbioni E, Marcomini A, Volpi Ghirardini A. Embryotoxicity of TiO2 nanoparticles to Mytilus galloprovincialis (Lmk). Mar Environ Res. 2013; 92:71–8. doi:10.1016/j.marenvres.2013.08.015.

[36] Chan WH, Shiao NH. Cytotoxic effect of CdSe quantum dots on mouse embryonic development. Acta Pharmacol Sin. 2008; 29(2):259–66. doi:10.1111/j.1745-7254.2008.00743.x.

[37] Di Guglielmo C, López DR, De Lapuente J, Mallafre JM, Suàrez MB. Embryotoxicity of cobalt ferrite and gold nanoparticles: a first *in vitro* approach. Reprod Toxicol. 2010; 30(2):271–6. doi:10.1016/j.reprotox.2010.05.001.

[38] Maisano M, Cappello T, Catanese E, Vitale V, Natalotto A, Giannetto A, Barreca D, Brunelli E, Mauceri A, Fasulo S. Developmental abnormalities and neurotoxicological effects of CuO NPs on the black sea urchin Arbacia lixula by embryotoxicity assay. Mar Environ Res. 2015; 111:121–7. doi:10.1016/j.marenvres.2015.05.010.

[39] Yoo MH, Rah YC, Choi J, Park S, Park HC, Oh KH, Lee SH, Kwon SY. Embryotoxicity and hair cell toxicity of silver nanoparticles in zebrafish embryos. Int J Pediatr Otorhinolaryngol. 2016; 83:168–74. doi:10.1016/j.ijporl.2016.02.013.

[40] Agarwal R, Domowicz MS, Schwartz NB, Henry J, Medintz I, Delehanty JB, Stewart MH, Susumu K, Huston AL, Deschamps JR, Dawson PE, Palomo V, Dawson G. Delivery and tracking of quantum dot peptide bioconjugates in an intact developing avian brain. ACS Chem Neurosci. 2015; 6(3):494–504. doi:10.1021/acschemneuro.5b00022.

[41] Cupaioli FA, Zucca FA, Boraschi D, Zecca L. Engineered nanoparticles. How brain friendly is this new guest? Prog Neurobiol. 2014; 119–120:20–38. doi:10.1016/j.pneurobio.2014.05.002.

[42] Wu T, Zhang T, Chen Y, Tang M. Research advances on potential neurotoxicity of quantum dots. J Appl Toxicol. 2016; 36(3):345–51. doi:10.1002/jat.3229.

[43] Sun Y, Zhang G, He Z, Wang Y, Cui J, Li Y. Effects of copper oxide nanoparticles on developing zebrafish embryos and larvae. Int J Nanomed. 2016; 11:905–18. doi:10.2147/IJN.S100350.

[44] van Gelder MM, van Rooij IA, Miller RK, Zielhuis GA, de Jong-van den Berg LT, Roeleveld N. Teratogenic mechanisms of medical drugs. Hum Reprod Update. 2010; 16(4):378–94. doi:10.1093/humupd/dmp052.

[45] Resende TP, Ferreira M, Teillet MA, Tavares AT, Andrade RP, Palmeirim I. Sonic hedgehog in temporal control of somite formation. Proc Natl Acad Sci U S A. 2010; 107(29):12907–12. doi:10.1073/pnas.1000979107.

[46] Ozbudak EM, Lewis J. Notch signalling synchronizes the zebrafish segmentation clock but is not needed to create somite boundaries. PLoS Genet. 2008; 4(2):e15. doi:10.1371/journal.pgen.0040015.

[47] Yabe T, Takada S. Molecular mechanism for cyclic generation of somites: lessons from mice and zebrafish. Dev Growth Differ. 2016; 58(1):31–42. doi:10.1111/dgd.12249.

[48] Ventre S, Indrieri A, Fracassi C, Franco B, Conte I, Cardone L, di Bernardo D. Metabolic regulation of the ultradian oscillator Hes1 by reactive oxygen species. J Mol Biol. 2015; 427(10):1887–902. doi:10.1016/j.jmb.2015.03.007.

[49] Liu XQ, Zhang HF, Zhang WD, Zhang PF, Hao YN, Song R, Li L, Feng YN, Hao ZH, Shen W, Min LJ, Yang HD, Zhao Y. Regulation of neuroendocrine cells and neuron factors in the ovary by zinc oxide nanoparticles. Toxicol Lett. 2016; 256:19–32. doi:10.1016/j.toxlet.2016.05.007.

[50] Schiavo S, Oliviero M, Miglietta M, Rametta G, Manzo S. Genotoxic and cytotoxic effects of ZnO nanoparticles for *Dunaliella tertiolecta* and comparison with SiO_2 and TiO_2 effects at population growth inhibition levels. Sci Total Environ. 2016; 550:619–27. doi:10.1016/j.scitotenv.2016.01.135.

[51] Boran H, Ulutas G. Genotoxic effects and gene expression changes in larval zebrafish after exposure to $ZnCl_2$ and ZnO nanoparticles. Dis Aquat Organ. 2016; 117(3):205–14. doi:10.3354/dao02943.

Chapter 5

Environmental Fate of Zinc Oxide Nanoparticles: Risks and Benefits

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Additional information is available at the end of the chapter

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Abstract

Zinc oxide nanoparticles (ZnO-NPs) are among nanoscale materials displaying exponentially growing production due to their applications in the field of cosmetology, medicine, as antibacterial agent and catalyst. The ZnO nanomaterials release into the aquatic ecosystems through domestic and industrial wastewaters has the potential to induce pernicious effects on fish and other organisms. Increasing concerns on the environmental hazard to aquatic biota have been highlighted by the toxic potential of some metal-based nanomaterials. Several characteristics of ZnO-NPs (e.g. size, shape, surface charge and agglomeration state) play a central role in biological effects such as genotoxic, mutagenic or cytotoxic effects. Overall, Zn bioaccumulation, histopathological, and hematological changes with oxidative and cellular stress have been reported in ZnO-NPs exposed animals.

This chapter provides an overview on applications of ZnO-NPs followed by a brief outline on methods of synthesis and characterization, and the current knowledge on the ZnO-NPs interaction with fish as they are valuable models in ecotoxicology, sensitive to many contaminants, representing a potential source of food for humans. This chapter intends to provide information for a critical overview of the pros and cons of using these particles, factors influencing their effects, and potential human health implications.

Keywords: zinc oxide nanoparticles (ZnO-NPs), nano-ecotoxicology, fish, human health

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1. Introduction

1.1. Applications of zinc oxide nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) are commonly used in several domains of human activity such as cosmetics, paints, optoelectronics, and pharmaceuticals, due to their low cost and interesting properties (e.g., conductivity, chemical stability, catalytic properties, photonics and optoelectronics, antibacterial, antifungal, and UV filtering properties). ZnO-NPs are highly used in the cosmetic industry, typically in sunscreens and facial creams [1]. In the biomedical field, ZnO-NPs have been applied in cell imaging [2, 3], drug delivery, and have demonstrated promising results in cancer research (for review see [4, 5]).

ZnO-NPs have shown to decrease the viability of cultured cell lines derived from human cancers. ZnO-NPs induced a 50% reduction in cell viability in MCF7 (breast cancer) and A549 (lung cancer) cell cultures, at a very low concentration (31.2 μg ml^{-1}), with size-dependent effectiveness [6]. A high toxicity on T98G (brain cancer) cells, moderate toxicity on KB (skin cancer) cells, and low toxicity on normal human HEK cells have also been reported [7]. ZnO-NPs have been proposed as genotoxic since they induced micronucleus in those cells. Apoptosis and intracellular production of reactive oxygen species (ROS) have been reported on melanoma cancer cells after exposure to different doses of ZnO-NPs [8]. These nanomaterials also exhibited activity against HepG2 (liver cells) cells depending on the dose [9]. A time-dependent reduction in the viability of murine cancer cells after exposure of ZnO-NPs was recently documented by [10]. ZnO-NPs strong protein adsorption properties may also lead to its use in other biomedical applications. ZnO-NPs may be used to modulate metabolism and cellular responses, and have been proven useful for the detection of low levels of biomarkers (e.g., proteins/peptides [11]). ZnO-NPs have shown promising results as cholesterol sensors, controlling diabetes and hyperglycemia, modulation of some allergic reactions, via inhibition of mast cell degranulation [12] as well in tissue engineering scaffolds to enhance angiogenesis [13].

As for other nanoparticles, ZnO-NPs may also be toxic for some microorganisms, making them potential antibacterial, antifungal, and antiviral agents. This is an important feature of these nanomaterials considering the increasing concerns related to the proliferation of pathogenic microorganisms that are multiresistant to conventional antibiotics. ZnO-NPs may interact with the bacterial surface and/or with the bacterial core, exhibiting different bactericidal mechanisms. Antimicrobial properties of ZnO-NPs have been demonstrated on bacteria such as *Bacillus subtilis, Escherichia coli* and *Pseudomonas fluorescens* [14], *Staphylococcus aureus* and *Salmonella typhimurium*, as well as on the fungi *Aspergillus flavus* and *Aspergillus fumigatus* [15]. In a study aiming to evaluate the immunological and antibacterial mechanisms of ZnO-NPs against human pathogens, Rashmirekha et al. [16] reported a higher effect of ZnO-NPs against *Staphylococcus aureus* when compared to *Mycobacterium bovis-BCG*. ZnO-NPs were able to disrupt bacterial cell membrane integrity, decrease cell surface hydrophobicity, and downregulate the transcription of oxidative stress-resistance genes in bacteria. The intradermal administration of ZnO-NPs reduced the skin infection, bacterial load, and inflammation in mice. ZnO-NPs treatment also increased the bacterial killing by inducing ROS. Virostatic

potential of micro-/nanofilopodia-like ZnO structures against herpes simplex virus-1 was reported by Mishra and colleagues [17]. Antoine and colleagues [18] synthesized 200 nm to 1 μm ZnO tetrapod-like structures, by flame transport method, to check the antiviral properties of ZnO micro- and nanostructures against HSV-2. ZnO tetrapods blocked the HSV-2 entry into the target cells and stopped the virus dispersal among already infected cells. The prophylactic treatment showed a decrease in HSV-2 internalization in both UV-treated and in nontreated conditions. The decreased internalization supports the preventive function of ZnO tetrapod nanoparticles against the ability of viruses to enter into the susceptible cells. ZnO tetrapods treatment decreased the cell fusion and syncytial formation of CHO-K1 cells.

ZnO-NPs synthesized by Nohynek et al. (2007), using wet chemical methods, revealed a high antibacterial activity, due to its inherent ability to absorb UV irradiation and optical transparency. This makes ZnO-NPs an important compound for the cosmetic industry, namely in formulations for sunscreens and facial creams [1]. The antibacterial activities of ZnO-NPs, as mentioned above, significantly contribute to its value in food processing industry, as a potent sanitizing agent for disinfecting and sterilizing food industry equipment and containers against foodborne pathogenic bacteria. ZnO-NPs are able to disrupt *E. coli* and *S. aureus* cell membrane causing cytoplasmic leakage and able to inhibit and kill the foodborne pathogens [19].

At industrial level, ZnO-NPs have various applications in catalysis and electronics [20]. ZnO-NPs can be used in infrared and chemical sensors, in the manufacture of rubber and cigarettes (used as filter) and preparation of creams and ointments used to treat skin diseases. The range of possible applications of ZnO-NPs also includes agriculture. Studies have shown potential beneficial effects of ZnO-NPs on seed germination, water purification, and soil remediation. Peanut seeds treated with 25 nm ZnO-NPs (1000 ppm) displayed high germination, seeding vigor, and plant growth [21], while decreased ryegrass germination has been reported after ZnO-NPs exposure [22]. Furthermore, the potential of ZnO-NPs to reduce microbial biomass and diversity [23] must also be taken into account.

1.2. Synthesis and characterization of ZnO nanoparticles

ZnO-based materials have been the subject of several reviews in the past years. A detailed survey on the literature concerning the synthesis and properties of nanosized ZnO can be found elsewhere [24]. ZnO is an inorganic crystalline compound with a band-gap energy located in the UV region that widens as the size of the particles decreases below a threshold of a few nanometers. Both in the bulk form and as a nanoscale material, ZnO is an important material for several applications including in electronics and optical devices [24]. ZnO-NPs may be synthesized by a variety of methods, selected based on the desired application, morphology, and size. Chemical and physical parameters (e.g., solvent type, precursors, pH, and temperature) are of high relevance in the synthesis protocols. A variety of shapes (nanorods, nanosphere, nanotubes, nanowires, nanoneedles, nanorings, spirals, drums, polyhedrons, disks, flowers, stars, boxes, and plates) may be produced, each displaying morphological-dependent physicochemical properties [25] that allow the exploitation of a variety of applications.

Some preparative methods include chemical vapor deposition, precipitation in aqueous solution, hydrothermal synthesis, sol-gel method, and synthesis using microemulsions and mechanochemical processes. These methods allow the production of particles differing in shape and size. Some available reviews present a thorough explanation of the principles and techniques involved in the different procedures [26]. Briefly, the mechanochemical method is based on high-energy dry milling; the controlled precipitation method involves hydrolysis of a Zn(II) solution, in conditions that limit uncontrolled growth of particles, eventually followed by a thermal treatment to improve crystallinity; the hydrothermal method is a simple and environmentally friendly technique that involves the thermal treatment of Zn(II) aqueous solutions under auto-generated pressure, by using an autoclave as the reaction vessel. ZnO-NPs have also been prepared using a Zn(II) precursor in the presence of plant extracts [27, 28] as cost-effective approaches, with promising results in terms of bioapplications. Selected examples of synthesis methods for ZnO-NPs are presented below.

ZnO-NPs were synthesized by Aneesh et al. [29] by hydrothermal treatment of $Zn(CH_3COO_2)$ $2H_2O$ (0.1 M) solutions. ZnO-NPs were prepared by varying the growth temperature and concentration of the Zn(II) precursor. X-ray diffraction (XRD) performed on powdered samples revealed nanoparticles of *wurtzite*-type structure. This synthesis yielded particles of sizes between 7 and 24 nm. Overall, particle size increased with growth temperature and decreased with concentration of precursor. Ramimoghadam et al. [30] synthesized ZnO-NPs also by a hydrothermal method, using palm olein as biotemplate. Different morphologies including flake-flower and 3D star-like structure were obtained. The concentration of palm olein has an effective role on observed morphological changes of the synthesized nanoparticles. These changes are possibly due to the reaction between carboxylic groups of palm olein and hydroxyl groups at the surface of ZnO. The biotemplates could be also used to modify the surface properties of ZnO-NPs.

Soni and Koser [31] used a hydrolysis method for the synthesis of ZnO-NPs, with different concentrations of a surface-protecting agent (thioglycerol). UV-VIS spectroscopy revealed blueshifts in the absorption bands of the samples, as compared to the spectrum of typical bulk ZnO, as an indication for the presence of nanosized ZnO. The absorption band edge was observed in the UV region at wavelength 365, 362, and 364 nm for ZnO-NPs synthesized using 0.12, 0.3, and 0.5 ml of capping agent, respectively. The samples were composed of particles with average sizes between 3.5 and 3.9 nm, depending on the amount of molar concentration of the capping agent. Increasing the concentration of capping agent, the average particle size decreased and the respective band gap widens due to quantum size effects [24]. Also using a hydrolysis method, Wang et al. [32] have synthesized nanometric ZnO using cetyltrimethylammonium bromide (CTAB) as surfactant. These authors reported high-crystalline nanoparticles of 50 nm average diameter. CTAB affected the process of nucleation and growth of crystallites during the synthesis also preventing the formation of ZnO agglomerates.

Giri et al. [33] synthesized hexagonal ZnO-NPs and nanorods by low-temperature oxidation of metallic Zn powder in the presence of acetic acid and trifluoroacetic acid. The final colorless powders were a first indication for the presence of ZnO. In this method, acetic acid and

trifluoroacetic acid induced the growth of hexagonal-type ZnO-NPs and ZnO nanorods, respectively, whose crystalline nature was confirmed by XRD. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images displayed hexagonal cross section of the nanoparticles and nanorods. The samples showed strong UV absorption peaked at 378 nm. A green synthesis method was used by Oudhia et al. [34] for ZnO nanotubes aiming biomedical application. For this purpose, neem leaf extract as biotemplate was used. The XRD pattern indicated the *wurtzite*-type structure of ZnO-NPs. The average crystalline size of the synthesized nanotubes was estimated as 25 nm by using Debye-Scherrer equation applied to the XRD patterns of the samples.

Barreto et al. [35] synthesized ZnO-NPs by microwave-assisted method and checked the effect of precursor reagent, temperature, irradiation time, and additives on the morphology of synthesized nanoparticles by using ($Zn(NO_3)_2 6H_2O$, $Zn(CH_3COO_2)2H_2O$, or $ZnCl_2$) as precursor. Radiation temperature of 80–140°C and increase in the irradiation time give high purity and homogenous size and shape of nanoparticles. The final pH is another important variable which causes significant changes in the morphology of the final particles. The addition of the anionic surfactant (AOT, sodium di-2-ethylhexyl sulfosuccinate) to the reaction medium allowed the synthesis of smaller particles. Kumar and Rani [36] synthesized ZnO-NPs by using microemulsions as nanoreactor for the synthesis of ZnO using $ZnSO_4$ salt. The stable reverse micelle microemulsion was prepared by mixing a nonionic surfactant, Triton X-100, PVP (used as co-stabilizing agent), cyclohexane, and distilled water. XRD diffraction analysis shows the typical hexagonal *wurtzite*-type structure of ZnO. TEM revealed nanoparticles of 10–12 nm in average size and rod shaped, and UV-VIS spectroscopy was use to estimate the optical band gap of the samples.

Tsuzuki and Cormick [37] synthesized nanocrystallites of ZnO of 26 nm size by a mechanochemical method using $ZnCO_3$ as the precursor. It was observed that a milling time of 4 h was enough for the synthesis of ZnO-NPs. Song et al. [2] synthesized ellipsoidal ZnO-NPs with high crystal quality by another mechanochemical method. It was observed that depending on the solvent used, the ZnO-NPs remained dispersed with a mean diameter of 21 nm (nonpolar solvents), whereas in more polar solvents the nanoparticles gradually aggregated to a diameter of about 200 nm. Photoluminescence spectra of ZnO-NPs have been reported.

The routine methodologies used to characterize ZnO-NPs (colloids and powders) are those commonly applied to characterize other types of nanoparticles and include dynamic light scattering (DLS) techniques, UV-VIS absorption spectroscopy, selected area electron diffraction (SAED), and powder X-ray diffraction (XRD). The size and shape of the nanoparticles are directly analyzed by using microscopy (transmission electron microscopy—TEM, atomic force microscopy—AFM, or scanning electron—SEM). The crystalline phase (typically *wurtzite* type) can be identified by using XRD and the surface charge of the colloidal NPs through zeta potential measurements. A number of processes involving NPs are mediated by the surface. Although its characterization is not straightforward, important information can be acquired by using Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy, and X-ray photoelectron spectroscopy (XPS) among other techniques.

2. Nanoparticles in aquatic systems

Increase in production and applications of ZnO-NPs is expected to result in its increased release into the environment. Ultimately, the aquatic ecosystems will probably be the main recipients, mainly as a result of industrial and domestic wastewaters [38]. As one of the most produced nanoparticles in the European Union, with an estimated production of around 1600 t [39], ZnO-NPs environmental release may occur as early as their production, production of products containing nanoparticles, during their use and end of life of those products. Despite the knowledge that nanoparticles are increasingly being used in different fields of human activity, the quantification of their release in the environment, at any given time, is quite challenging due to the limited data on their current and expected prevalence in commercial products [40–42]. The technical difficulties associated with quantification of ZnO-NPs levels in the environment led to the need to predict environmental concentrations based on market penetration of nanomaterials, known usage of the products as well as fate/behavior. According to the available data, the theoretically predicted average environmental concentration of ZnO-NPs in European surface waters is 0.09 $\mu g\ L^{-1}$ (with 85% confidence intervals: 0.05–0.29) [39]. No standards have yet been established for permissible levels of nanoparticles in the environment. Nonetheless, in addition to dose, physicochemical properties of nanoparticles (e.g., size, shape, chemical composition, aggregation) as well as ionic strength and pH of receiving media play determinant roles on their behavior, bioavailability, and biological effects of nanoparticles [43]. Once released into the environment, ZnO-NPs may display different behaviors. Nanoparticles in the environment may stay in suspensions as individual particles; dissolve; aggregate; form larger particles and ultimately sediment; adsorb onto water constituents (e.g., dissolved organic matter); transform chemically (e.g., due to redox reactions) or biologically (e.g., in the presence of microorganisms) in the marine environment [44]. Once in the environment, most ZnO-NPs are likely to precipitate due to its poor colloidal stability [45]. The available studies indicate that aggregation of ZnO-NPs, as for other nanoparticles, increases with ionic strength. In high ionic strength environments, reduction in electrostatic repulsion forces between the nanoparticles occurs, promoting aggregation and sedimentation. However, the presence of natural substances such as humic acids may help to steric and electrostatic stabilization of ZnO-NPs, aiding in their transport, mobility, and dispersion [46]. Other highly relevant alterations that may occur in the environment and lead to toxic effects of ZnO-NPs are the dissolution and redox transformations. ZnO-NPs may dissolve, releasing Zn ions which may induce toxic effects [47], with reported faster dissolutions at smaller sizes [48]. Redox reactions on ZnO-NPs' surface may lead to the production of ROS, which are able to oxidize organic compounds and lead to oxidative stress. Thus, for risk assessments of nanoparticles such as ZnO-NPs, different factors have to be taken into account based on the wide variety of reactivities and properties of a particular type of nanoparticle [49].

The available studies on the behavior of ZnO-NPs in water systems have been performed under laboratory conditions, focusing mostly on freshwater. Available data indicate that dissolution is dependent on concentration, with the lowest dissolution percentage at the highest ZnO concentrations [50].

3. Interaction of nanoparticles with aquatic organisms

Among aquatic species, fish have been considered as the ideal sentinels to detect toxicological effects, due to their wide distribution, known physiology, and sensitivity to exposure to contaminants via food or water. In this chapter, some of the available studies with fish at different life stages (embryos, juveniles, and adults) are presented in **Table 1**.

Primary particles	Study aim	Test organisms	Exposure protocol	Assessed endpoints	Main effects	References
Size: <100 nm	To assess developmental toxicity, oxidative stress, and DNA damage	*Danio rerio* (Zebrafish)	Waterborne exposure to 1, 5, 10, 20, 50, and 100 mg L^{-1} up to 144 h postfertilization	Embryo/larvae survival, hatching, and malformation rates; ROS measurement; DNA damage; antioxidant enzymes; lipid peroxidation mRNA levels of genes encoding antioxidant proteins and regulation of ROS production	Reduction of hatching rate and induction of malformations; ROS generation; DNA damage	[51]
Size: 25 nm	To identify potential mechanisms of cardiorespiratory effects of ZnO-NPs and characterize the ecophysiological importance of ZnO-NPs toxicity	*Catostomus commersonii* (White sucker)	Waterborne exposure for 25 h to 10 mg L^{-1}; for 15 and 30 h to 1 mg L^{-1}	Gill morphology, cardiorespiratory function	Damage to the gill epithelium; decreased heart acetylcholinesterase activity; reduction of aerobic capacity	[52]
Size: 25 nm	To assess the effects of ZnO-NPs exposure in the liver of a freshwater fish	*Catostomus commersonii* (White sucker)	Waterborne exposure for 29.5 h to 1 mg L^{-1}	Biomarkers of oxidative stress and antioxidant response	Changes in levels of hepatic enzyme activities, antioxidants, and	[53]

Primary particles	Study aim	Test organisms	Exposure protocol	Assessed endpoints	Main effects	References
					lipid peroxidation products	
Size: 20 nm	To study stress proteomic responses	*Oryzias melastigma* (Medaka fish)	Waterborne exposure to 4 and 40 mg L^{-1} for 96 h	Molecular biomarkers (SOD, MT and HSP70)	Upregulation of HSP70	[54]
Size: 30 nm	To evaluate bioaccumulation and subacute toxicity compared with bulk particles	*Cyprinus carpio* (Juvenile carp)	Waterborne exposure (5–1000 mg L^{-}) for 30 days	Accumulation in different tissues (gill, liver, intestine, muscle, and brain); histopathological changes; enzyme activities (e.g., Na^{+}/K^{+}ATPase, and SOD) nonenzymatic antioxidants and oxidative damage	Higher bioaccumulation, oxidative effect, and histopathological changes than bulk ZnO	[55]
Size: 30 nm	To study Zn accumulation and the mechanism of hepatic detoxification in comparison with bulk ZnO and Zn^{2+}	*Carassius auratus* (Gold fish)	Waterborne exposure for 30 days to 2 mg L^{-1}	Zn concentration and its subcellular distribution gills, liver, gut, and muscle	Tissue-specific bioaccumulation dependent on the exposed material	[56]
Size: 15–350 nm	To compare the effects of zinc compounds in the form of nano-, microparticles and ions	*Brachydanio* (Danio) *rerio* (zebrafish)	Waterborne exposure to 0.2, 2, 10 and 20 mg L^{-1} for 120 h	Embryo/larvae survival, hatching, and malformation rates	Retardation of hatching and deviations in embryonic development; adherence of the particles on the egg surface at high ZnO-NPs concentrations	[57]
Size: 50–60 nm	To compare the effect of different	*Ctenopharyngodon idella* (Grass carp)	Diet exposure (4% body weight	Lethality, growth performance, food	Improvement in growth performance	[58]

Primary particles	Study aim	Test organisms	Exposure protocol	Assessed endpoints	Main effects	References
	forms zinc (ZnO, $ZnSO_4$, ZnO-NPs) on growth and hematological indices		for 90 days	conversion ratio and efficiency, hepatosomatic index; blood parameters	and red blood cell count with supplementation with ZnO-NPs compared to oxide and sulfate form	
Size: <100 nm	To evaluate the acute toxicity and hematological effects	*Oreochromis mossambicus* (Tilapia)	Waterborne exposure to 30, 50 and 70 mg L^{-1} for 96 h	Blood parameters	96 h LC_{50} of ZnO-NPs between 100 and 110 mg L^{-1}; chromosomal damages, changes in blood parameters	[59]
Size: <100 nm	To evaluate the long-term effects of 3 sublethal concentrations	*Cyprinus carpio* (Carp)	Exposure for 21 days of three sublethal concentrations from the 96 h LC50 value (4.897 mg L^{-1})	Histopathological changes in the liver	Dose-dependent histological alterations generally associated with the response of hepatocytes to toxicants	[60]
Size: <100 nm	To study the acute toxicity (LC_{50}) and gill histopathology	*Cyprinus carpio* (Carp)	Waterborne exposure for 96 h to 2, 4, 8, and 16 mg L^{-1}; waterborne exposure to sublethal concentrations for 21 days	Lethality; gill histopathology	A 96 h LC_{50} of 4.897 mg L^{-1}. Histopathological alterations in the gills at sublethal concentrations at higher concentrations	[61]
Size: 30 nm	To quantify the trophic transfer of ZnO-NPs by feeding *D. rerio* with *D. magna* exposed to ZnO-NPs prior to the feeding experiments	*Danio rerio* (Zebra fish) and *Daphnia magna* (Water flea)	Fish exposure through diet for 14 days to *D. magna* (4–5 days old) preexposed to ZnO-NPs and ZnO-octyl NP (1.0 mg Zn L^{-} for 24 h)	Zn content on fish body burden	Uptake of both ZnO-NPs and ZnO-octyl NP with values exceeding by tenfold the levels obtained through aqueous exposure in other studies	[62]

Primary particles	Study aim	Test organisms	Exposure protocol	Assessed endpoints	Main effects	References
Size: 50 nm	To determine lethal concentration and histopathological lesions	*Cyprinus carpio* (Carp)	Waterborne exposure for 24 h to 10–50 mg L^{-1}	Lethal concentrations and histopathological alterations in gills, liver, kidney, and pancreas	Histopathological lesions in the kidney and gills; necrosis in liver, and hemorrhage in pancreas	[63]

Abbreviations: HSP70: heat shock protein 70; MT: metallothionein; NPs: nanoparticles; ROS: reactive oxygen species; SOD: superoxide dismutase.

Table 1. Examples of research studies on the effects of ZnO-NPs on fish.

As shown in **Table 1**, examples of recent research on ZnO-NPs interaction with fish have essentially focused on freshwater organisms such as *Cyprinus carpio, Oreochromis mossambicus, Tilapia zillii, Oreochromis niloticus, Ctenopharyngodon idella, Carassius auratus*, and *Danio rerio*. Several techniques and approaches were used in those studies including, mainly, analytical methods for detection of metal in tissues as well histopathological, hematological, and oxidative stress endpoints to study the effects of ZnO-NPs, and histopathological, hematological, and oxidative stress as relevant endpoints to evaluate the possible interrelationships.

The effects of ZnO-NPs have been associated with two main mechanisms: oxidative stress and nanoparticle-protein interactions [52]. Although some of the effects have been associated with the release of Zn ions as a result of particle dissolution, not all studies confirm that the toxic effects are due to dissolution. Higher bioaccumulation and effects have been observed after exposure to nanoparticle form when compared to bulk form, confirming the complexity and specificity of mechanisms associated with experimental conditions. Nonetheless, it is clear that more studies are needed, with lower concentrations and longer exposure periods, representing a more environmentally relevant scenario. There is also a clear need for studies on combined effects of abiotic factors variation (e.g., temperature, salinity, UV radiation), classical environmental contaminants (e.g., organic compounds, pesticide and pharmaceuticals), and ZnO-NPs, to represent an environmentally relevant scenario. There is also a need to assess the effects of ZnO-NPs in organisms present in high ionic strength environment (e.g., estuaries and marine environments). The information obtained in trophic transfer studies supports the concerns of potential effects of nanoparticles, to higher trophic levels in which humans may also be a target.

Considering the tested endpoints, the available data revealed that histological and hematological responses occur. After ZnO-NPs exposure, both juvenile and/or adult fish have shown its accumulation on tissues such as brain, liver, muscle, and gills [55, 56]. Hao et al. [55] reported ZnO-NPs accumulation on tissues of juvenile carp (*Cyprinus carpio*), and cellular oxidative stress response was denoted as the main toxic mechanism of nano-ZnO. The bioaccumulative

behavior of ZnO-NPs and their potential trophic transfer from *Daphnia magna* to zebrafish (*Danio rerio*) was reported by Skjolding et al. [62].

Several parameters such as hematological approaches have been used for the monitoring of health conditions of fish [58, 59]. Blood parameters as red and white blood cells count, hemoglobin content, and hematocrit value, and red blood cell indices are usually assessed on some toxicological studies. Although enhancement on red blood cell count was reported on the grass carp *Ctenopharyngodon idella* with supplementation of NPs from [58], deleterious changes on blood parameters were documented on Tilapia *Oreochromis mossambicus* exposed for 96 h to ZnO-NPs (100–110 mg L^{-1}) [59].

Additionally, histology is also an important tool for the evaluation of fish health, showing the initial signs of lesions not easily noticeable during the macroscopic observation of tissues and organs [55]. Gills are vital organs for respiration and osmoregulation. Gill histopathological alterations can be considered as indicators of the ZnO-NPs–induced toxicity in the common carp [60]. Among other relevant organs, hepatic histopathological changes were documented as a result of exposure to ZnO-NPs [55, 61, 63].

As reported in **Table 1**, the ecotoxicological impacts of ZnO-NPs on fish include in most studies histopathological, hematological, and oxidative stress under different doses, protocols, and exposure [53, 55]. Tomilina et al. [57] reported decreased motility and increased curvature of tail in *Brachydanio* (*Danio*) *rerio* embryos exposed for 24 h to 0.01 mg L^{-1} ZnO-NPs and affected dynamics of hatching of *Brachydanio* (*Danio*) *rerio* prelarvae at higher concentrations.

In contrast, scarce data have demonstrated positive biological effects of ZnO-NPs. Reports on fish (*C. idella*) growth performance improvement after 90 days ZnO-NPs exposure, through diet, compared to oxide and sulfate form of Zn were recently published [58], suggesting a potential application of these particles on aquaculture.

4. Potential impacts on public health

The hazard potential of ZnO-NPs to humans in comparison with microparticulate and dissolved Zn has been evaluated in the context of major accident prevention [64]. Based on the analysis of endpoints of subtoxic events (inflammation, oxidative stress response, or gene expression profiling) over different timescales, the authors concluded that the hazard potentials of nano- and microparticles of ZnO are identical during acute (medium) and chronic (low) toxicity. Inhalation of ZnO fume and dust over the permissible exposure limit of 5 mg m^{-3} appears to be the riskiest toxic exposure, since Zn fume fever could be lethal [65]. ZnO is quite soluble in acids and alkalies, thereby the toxicity of ZnO-NPs was also compared to that of zinc(II) ions. When considering the concentration of dissolved Zn, no significant differences between exposure to ZnO and ZnO-NPs have been found in EC_{50} and LC_{50} values, using *Daphnia* and fish, respectively, as testing organisms. Clinical reports on human intoxication by ZnO-NPs are hardly any in the literature. Conversely, information regarding the toxicity of Zn ions for humans has been gathered during the last 60 years, since the first clinical reports on Zn fume fever [66].

Zn is an essential trace element and micronutrient for humans. Under physiological conditions, it exists as a redox neutral divalent cation that is reactive as a Lewis acid. The continuous external supply of this metal ion is vital for many metabolic pathways, given that there is no body storage depot for zinc and its exchange between tissues is limited. The man body Zn content is approximately 2 g, and the recommended daily intake is 8–11 mg (tolerable upper intake level, 40 mg day^{-1}). Bones and skeletal muscles contain more than half of the total body Zn, while the highest concentrations (>1 mg g^{-1} dry weight) are achieved in prostate gland [67]. Around 0.1% of the total body zinc is replenished daily through diet, and it is equivalent to the percentage that is hold in blood serum (90 μg dL^{-1}) [68].

Zinc homeostasis is tightly controlled at the whole body down to the subcellular level. In cells, half of the Zn content is in the cytoplasm, while nucleus and plasma membrane accounts for, respectively, 35 and 10% of the total cellular Zn [69]. Since this metal ion is mainly bound to proteins (i.e., metallothioneins) and sequestered in organelles (i.e., mitochondria, endoplasmic reticulum, Golgi apparatus, secretory granules, and other vesicular compartments), the cytosolic free Zn concentration is in the picomolar/nanomolar range. In the cytosol, Zn concentrations fluctuate in wave and spark manners, of which regulatory mechanism still not completely understood. Zinc transporters (ZnTs) are of outstanding importance for the cellular and subcellular zinc homeostasis (**Table 2**), since ions cannot be synthesized or broken down by cells. ZnT transporters (ZnT1-ZnT10) belong to the Solute Carrier Family 30A (SLC30A). This protein "Family" also comprises another group of proteins that translocate Zn across membranous barriers, the ZIP transporters (ZIP1-ZIP14). However, ZIP transporters are involved not only in Zn transport but also in the homeostasis of cadmium, manganese, iron, and calcium [70].

Transporters		
Name	**Main functions at subcellular level**	**Entries: Protein/Gen***
ZnT1	Zn^{2+} efflux through plasma membrane. Negative regulation of Zn^{2+} and Ca^{2+} transmembrane import and neurotransmitter secretion	Q9Y6M5 (ZNT1_HUMAN)/ SLC30A1
ZnT2 (2 isoforms)	Zn^{2+} transmembrane transport (accumulation in endosomes, lysosomes, and secretory vesicles in mammary epithelial cells). Regulation of sequestering of and response to Zn^{2+}	Q9BRI3 (ZNT2_HUMAN)/ SLC30A2
ZnT3	Zn^{2+} transporting ATPase (accumulation in synaptic vesicles, late endosomes, and lysosomes). Regulation of sequestering of and response to Zn^{2+}	Q99726 (ZNT3_HUMAN)/ SLC30A3
ZnT4	Zn^{2+} transmembrane transport (transport out of the cytosol—accumulation in endosomes, lysosomes, secretory vesicles, and trans-Golgi network)	O14863 (ZNT4_HUMAN)/ SLC30A4
ZnT5 (4 isoforms)	Zn^{2+} transmembrane transport into lumens of the Golgi apparatus and early compartments of the secretory pathway such as COPII-coated vesicles (putative transporter of Zn^{2+} into β cells in order to form insulin crystals). Required with	Q8TAD4 (ZNT5_HUMAN)/ SLC30A5

Transporters		
Name	**Main functions at subcellular level**	**Entries: Protein/Gen***
	ZnT7 for the activation of Zn-requiring enzymes, alkaline phosphatases, and ZnT6 and ZnT7 for the activation of TNAP	
ZnT6 (4 isoforms)	Zn^{2+} efflux transporter that allocates it to trans-Golgi network and vesicular compartment. Regulation of sequestering of and response to Zn^{2+}	Q6NXT4 (ZNT6_HUMAN)/ SLC30A6
ZnT7	Zn^{2+} transmembrane transport into lumens of Golgi apparatus and vesicular compartments. Required for activation of alkaline phosphatases and with ZNT5 and ZNT6 for the activation of TNAP	Q8NEW0 (ZNT7_HUMAN)/ SLC30A7
ZnT8 (4 isoforms)	Zn^{2+} efflux transporter which allocates it to intracellular vesicles (i.e., accumulation into insulin granules in pancreatic β cells, providing Zn^{2+} to insulin maturation and/or storage). Regulation of sequestering of and response to Zn^{2+}. Responsiveness to glucose, γ-interferon, and interleukin-1	Q8IWU4 (ZNT8_HUMAN)/ SLC30A8
ZnT9	Role in the p160 coactivator signaling pathway that mediates transcriptional activation by nuclear receptors. Transcriptional activation of Wnt-responsive genes	Q6PML9 (ZNT9_HUMAN)/ SLC30A9
ZnT10 (3 isoforms)	Zn^{2+} transmembrane transport into Golgi apparatus and early endosomes. Regulation of sequestering of and response to Zn^{2+}	Q6XR72 (ZNT10_HUMAN)/ SLC30A10
ZIP1 (2 isoforms)	A major Zn^{2+} uptake transporter in many cells; responsible for the rapid uptake and accumulation of physiologically effective Zn in prostate cells	Q9NY26 (S39A1_HUMAN)/ SLC39A1
ZIP2 (2 isoforms)	Zn^{2+} transport through the plasma membrane (uptake mediated by Zn^{2+}-HCO_3^- symport). It is involved in contact inhibition of normal epithelial cells, and loss of its expression is related to tumorigenesis	Q9NP94 (S39A2_HUMAN)/ SLC39A2
ZIP3 (2 isoforms)	Zn^{2+} transport through the plasma membrane (influx to cytosol). It is involved in cell morphogenesis and T cell homeostasis	Q9BRY0 (S39A3_HUMAN)/ SLC39A3
ZIP4 (2 isoforms)	Zn^{2+} transmembrane transport (influx to cytosol) It is involved in the regulation of cellular Zn homeostasis in response to Zn^{2+} availability (cycles between endosomal compartments and the plasma membrane)	Q6P5W5 (S39A4_HUMAN)/ SLC39A4
ZIP5	Zn^{2+} transmembrane transport (serosal to mucosal) through basolateral cell membrane in polarized cells	Q6ZMH5 (S39A5_HUMAN)/ SLC39A5
ZIP6 (2 isoforms)	Zn^{2+} transport through the plasma membrane (influx to cytosol)	Q13433 (S39A6_HUMAN)/ SLC39A6
ZIP7	Zn^{2+} transmembrane transport from the endoplasmic reticulum/Golgi apparatus to the cytosol that is stimulated by growth factors (EGF), $Ca^{2+}$and exogenous Zn^{2+}	Q92504 (S39A7_HUMAN)/ SLC39A7

Transporters		
Name	**Main functions at subcellular level**	**Entries: Protein/Gen***
ZIP8 (3 isoforms)	Zn^{2+} transport through the plasma membrane and endosomal and lysosomal membranes (influx and release to cytosol)	Q9C0K1 (S39A8_HUMAN)/ SLC39A8
ZIP9 (3 isoforms)	Zn^{2+} transport through the plasma membrane and trans-Golgi network membrane (influx and release to cytosol)	Q9NUM3 (S39A9_HUMAN)/ SLC39A9
ZIP10 (2 isoforms)	Zn^{2+} transport through the plasma membrane (influx to cytosol). Positive regulation of B cell proliferation, B cell receptor signaling pathway, and protein tyrosine phosphatase. Negative regulation of B cell apoptotic process	Q9ULF5 (S39AA_HUMAN)/ SLC39A10
ZIP11 (3 isoforms)	Zn^{2+} transport through the trans-Golgi network membrane (release to cytosol)	Q9NUM3 (S39A9_HUMAN)/ SLC39A9
ZIP12 (5 isoforms)	Zn^{2+} transport at the plasma membrane, nucleus, and Golgi apparatus (influx and release to cytosol). Regulation of microtubule polymerization, neuron projection development, and signal transduction	Q504Y0 (S39AC_HUMAN)/ SLC39A12
ZIP13 (2 isoforms)	Zn^{2+} transmembrane transport in the Golgi apparatus (release to cytosol)	Q96H72 (S39AD_HUMAN)/ SLC39A13
ZIP14 (3 isoforms)	Zn^{2+} transport through the plasma membrane (influx to cytosol). Broad-scope metal ion transporter with a preference for Zn^{2+} uptake (cellular uptake of nontransferrin-bound Fe)	Q15043 (S39AE_HUMAN)/ SLC39A14

Zn-finger proteins		
Family; subfamily	**Representative protein and its function**	**Protein entry***
ZNF593/ BUD20 C2H2	Zinc finger protein 593 negatively modulates the transcriptional regulatory activity of Oct-2	O00488
Teashirt C2H2	Teashirt homolog 2 is a putative transcriptional regulator in developmental processes, acting as transcriptional repressor	Q9NRE2
Sp1 C2H2	Transcription factor Sp9 positively regulates FGF8 expression in the apical ectodermal ridge and contributes to limb outgrowth in embryos	P0CG40
Snail C2H2	Transcriptional repressor scratch 1 binds E-box motif CAGGTG and modulates the basic helix-loop-helix transcription factors during neuronal differentiation	Q9BWW7
Sal C2H2	Sal-like protein 4 is an important transcription factor in the maintenance and self-renewal of embryonic and hematopoietic stem cells	Q9UJQ4

Zn-finger proteins		
Family; subfamily	**Representative protein and its function**	**Protein entry***
Odd C2H2	Protein odd-skipped-related 1 is a transcription factor in the regulation of embryonic heart and urogenital development	Q8TAX0
Krueppel C2H2; ZFX/ZFY	Zinc finger Y-chromosomal protein is a transcriptional activator through binding to the consensus sequence 5′-AGGCCY-3′	P08048
Krueppel C2H2; ZFP57	Zinc finger protein 57 homolog is a transcription regulator that binds to a 5′-TGCCGC-3′ consensus sequence and recognizes the methylated CpG within this element. It is important for the maintenance of maternal and paternal gene imprinting through control of DNA methylation during the earliest multicellular stages of development at multiple imprinting control regions	Q9NU63
Krueppel C2H2; ZBTB18	Zinc finger and BTB domain-containing protein 18 is a transcriptional repressor that binds to the consensus DNA sequence 5′-[AC]ACATCTG[GT][AC]-3′ containing E-box core. It is involved in the recruitment of chromatin remodeling multiprotein complexes, the regulation of skeletal myogenesis, progenitor cell division, and postmitotic cortical neurons survival	Q99592
Krueppel C2H2; Hic	Hypermethylated in cancer 1 protein is a transcriptional repressor that recognizes and binds to the consensus sequence ‘5-[CG]NG[CG]GGGCA[CA]CC-3’. It regulates the Wnt signaling pathway, p53/TP53-dependent apoptotic DNA damage responses, and the transcription of CCND1/cyclin-D1 and CDKN1C/p57Kip2 in quiescent cells. May act as a tumor suppressor and is involved in development of head, face, limbs, and ventral body wall	Q14526
Krueppel C2H2	Krueppel-like factor 1 is a transcription regulator of erythrocyte development and switch factor during erythropoiesis. When sumoylated, acts as a transcriptional repressor by promoting interaction with CDH2/MI2β and represses megakaryocytic differentiation	Q13351
Ikaros C2H2	DNA-binding protein Ikaros has transcription regulator activity, via binding to γ-satellite DNA, which is isoform-specific and modulated by dominant-negative inactive isoforms. It increases normal apoptosis in adult erythroid cells and confers early temporal competence to retinal progenitor cells	Q13422
GLI C2H2	Both isoforms of zinc finger protein GLI1 are transcriptional activators that bind to the DNA consensus sequence 5′-GACCACCCA-3′, but activate different sets of genes. Isoform 1 plays a role in cell proliferation and differentiation, through SHH signaling pathway, whereas isoform 2 activates CD24 expression. Promotes cancer cell migration	P08151
EGR C2H2	E3 SUMO-protein ligase EGR2 is a transcription factor that binds to two sequence-specific DNA sites located in the promoter region of HOXA4. Supports SUMO1 conjugation to	P11161

Zn-finger proteins

Family; subfamily	Representative protein and its function	Protein entry*
	coregulators NAB1 and NAB2, whose sumoylation downregulates EGR2 own transcriptional activity	
DZIP C2H2	Zinc finger protein DZIP1 interaction with DAZ supports the participation in spermatogenesis and primary cilia formation through Hedgehog signaling pathway	Q86YF9
Delta-EF1/ ZFH-1 C2H2	Zinc finger E-box-binding homeobox 1 is a transcriptional repressor that positively regulates neuronal differentiation and promotes tumorigenicity	P37275
CTCF	Transcriptional repressor CTCF plays important roles in gene silencing, chromatin remodeling, interchromosomal association, regulation of epigenetic modifications, oocyte and preimplantation embryo development. It is also a putative tumor suppressor	P49711
AEBP2/jing C2H2	Zinc finger protein AEBP2 is a DNA-binding transcriptional repressor that stimulates PRC2 complex activity	Q6ZN18

Enzymes

Recommended name	EC number**	Comments
D-Lactate dehydrogenase (acceptor)	1.1.99.6	Alanine metabolism
Formaldehyde dismutase	1.2.98.1	Contains a tightly but noncovalently bound NADP(H) cofactor, as well as Zn^{2+} and Mg^{2+}
Peptide-methionine (R)-S-oxide reductase	1.8.4.12	Selenoprotein. Prevention of oxidative stress damage caused by reactive oxygen species by reducing the oxidized form of methionine back to methionine and thereby reactivating peptides that had been damaged
Superoxide dismutase	1.15.1.1	Degradation of reactive oxygen species and superoxide radicals
Histone acetyltransferase	2.3.1.48	Different specificities toward histone acceptors
RING-type E3 ubiquitin transferase	2.3.2.27	Degradation of misfolded protein
Protein geranylgeranyltransferase type I	2.5.1.59	Zn metalloenzyme. Zn^{2+} is required for peptide, but not for isoprenoid, substrate binding. Inhibition induces simultaneous p53-dependent apoptosis and autophagy in airway smooth muscle cells
Tyrosine transaminase	2.6.1.5	Involved in multiple metabolic pathways
Riboflavin kinase	2.7.1.26	Mg^{2+} is preferentially required for activity. Essential in recruiting Nox1 to death receptor4/5, critical role in the KD548-

Enzymes		
Recommended name	**EC number****	**Comments**
		Fc-mediated reactive oxygen species accumulation and coupling of TNF-receptor-1 to NADPH oxidase
Rhodopsin kinase	2.7.11.14	Inhibited by Zn^{2+}
β-Adrenergic-receptor kinase	2.7.11.15	Inhibited by Zn^{2+}
tRNase Z	3.1.26.11	Involved in both, nuclear and mitochondrial tRNA 39 end maturation and in the p53 signaling pathway
N-acetylphosphatidylethanolamine-hydrolyzing phospholipase D	3.1.4.54	Contains Zn^{2+} and is activated by Mg^{2+} or Ca^{2+}. It does not hydrolyze phosphatidylcholine and phosphatidylethanolamine
Aminopeptidase B	3.4.11.6	Exopeptidase strictly specific for the removal of N-terminal basic residues from peptides and proteins
Xaa-Trp aminopeptidase	3.4.11.16	Zn^{2+}containing glycoprotein from renal and intestinal brush border membranes
Aminopeptidase I	3.4.11.22	Activity is stimulated by both Zn^{2+} and Cl^{-}
N-acyl-aliphatic-L-amino acid amidohydrolase	3.5.1.14	Contains Zn^{2+} (completely inactivated by metal removal, whereas addition of Zn^{2+}, Mn^{2+}, or Fe^{2+} restores activity). It is involved in the hydrolysis of *N*-acylated or *N*-acetylated amino acids (except L-aspartate)
Cu^{2+}-exporting ATPase	3.6.3.4	Zn binds with a stoichiometry of 6–1 and induces a conformational change in the N-terminal domain that is different from those observed for Co binding, leading to a loss of secondary structure in the domain
Mitochondrial protein-transporting ATPase	3.6.3.51	A nonphosphorylated, non-ABC (ATP-binding cassette) ATPase involved in the transport of proteins or preproteins into mitochondria using the TIM protein complex
Porphobilinogen synthase	4.2.1.24	Contains Zn^{2+} at the active site. Essential for respiration and a primary target in Pb intoxication
Ubiquitin-protein ligase	6.3.2.19	Crucial role in the recognition and degradation of target proteins by 26S proteasomes

*UniProtKB/Swiss-Prot—European Bioinformatics Institute.
**Enzyme Database—BRENDA and IUBMB Enzyme Nomenclature.

Table 2. Illustrative examples of human Zn transporters, Zn-finger proteins, and enzymes that require Zn.

Zn ions are important for the regulation of central biochemical processes (gene transcription and the metabolism of lipids, proteins, and nucleic acids), which impacts a variety of physiological functions (e.g., neuronal, endocrine, skeletal, reproductive, immune, and healing). It is estimated that the 10% of the proteins (around 3000 proteins) encoded in the human genome are zinc proteins [71]. According to the last release of the UniProtKB/Swiss-Prot database, more than one hundred of human proteins are zinc finger (ZnF) macromolecules (**Table 2**). Apart from the structural role of Zn in ZNFs [72], which contributes to shape the zinc-binding repeats as molecular scaffolds for tight binding of their target molecules (DNA, RNA, other proteins, or lipids), this metal ion is also essential for enzyme catalysis and cell signaling (**Table 2**) [73, 74]. In enzyme catalytic centers, Zn often promotes substrate activation by stabilizing negative charges due to strong Lewis acid properties. The metal ion acts as endocrine, paracrine, autocrine, and intracrine mediator. In cells, the distribution of Zn is modified by the stimulation that triggers its release in the central nervous and neuroendocrine systems. Zn is also a ubiquitous cytosolic second messenger, leading to fast alteration of signaling enzyme activities (i.e., phosphodiesterases, mitogen-activated protein kinase, protein kinase C, protein tyrosine phosphatases, calcineurin, caspases) and afterward to the biosynthesis of proteins that control its cytosolic concentrations. Interestingly, Zn accumulation in specific subcellular compartments appears to occur during both physiological and pathological conditions. For instance, the Zn spark that follows the calcium wave during fertilization is thought to be crucial for further cell cycle resumption in eggs [75]. Accumulation of Zn in lysosomes is a common observation during neurodegenerative processes and intoxication [76].

In humans, symptoms of Zn deficiency include: severe anemia, persistent diarrhea, immune insufficiency ensuing recurrent inflammations and impairment of wound healing, growth retardation, hypogonadism, skin and eyes abnormalities, baldness mental lethargy, brain dysfunctions, and behavioral changes. Zn deficiency still continues a global public health concern, particularly in developing countries where it causes mortality among young children [77]. Conversely, minor Zn deficiency among elderly population and individuals who undergo gastric bypass surgery for obesity seems to be increasing in industrialized countries. Thereby, development of high efficient Zn-enriched nutritional supplements could be advantageous in decreasing the incidence of degenerative and immunodeficiency disorders, infections, and persistent diarrhea. Current Zn formulations better absorbed through supplements are in the form of picolinate or chelates of amino acids. Duodenum is the principal site for Zn absorption. Therefore, intestinal pathologies that cause poor micromineral absorption, such as Crohn's disease, can also induce Zn malabsorption. Other metal ions, such as Ca^{2+}, Fe^{2+}, and mostly Cu^{2+} compete with Zn^{2+} during translocation from the apical surface of the villae to the basolateral surface of enterocytes. In matter of fact, Zn intake close to the recommended dietary allowance (15 mg/day) may cause copper and iron deficiency and adversely affect HDL cholesterol concentrations. Overt symptoms of Zn poisoning (i.e., nausea, vomiting, epigastric pain, lethargy, and fatigue) usually occur only after exposure to extremely high Zn levels [78].

Zn-related diseases might be prompted either when its scarcity and overload go beyond the limited cellular Zn buffering capacity, which seems to be rather sensitive to environmental factors. Apparently, healthy individuals tolerate up to 10-fold changes of the recommended

daily intake of Zn [79]. Whether exposure to Zn concentrations outside the physiological range promotes salutary or injurious effects depends on Zn concentration. Different ranges of Zn concentration appear to be required for exhibiting beneficial properties as antioxidant, antiinflammatory, and antiapoptotic agent [80]. Intriguingly, both Zn excess and scarcity undermine the loosely equilibrium toward prooxidant, proinflammatory and proapoptotic actions.

Lung toxicity after inhalation of ZnO-NPs is likely the most documented deleterious effect in the literature. The severity of ZnO-NPs-induced inflammatory condition is significantly correlated with the mass and surface area of the nanoparticles, suggesting that the toxic effect of ZnO-NPs is mainly caused by the release of Zn ions [81]. It is also claimed that intact ZnO-NPs have a unique way of inducing inflammatory effects compared with dissolved Zn ions [82]. For example, ZnO-NPs may either stimulate the production of IFN-γ and subsequent macrophage activation, neutrophilic infiltration, and fibrosis (Th1 inflammatory response) or cause a mixed inflammatory cell immune response by triggering a Th2 response [83]. Inhaled ZnO-NPs, through the olfactory bulb–brain translocation pathway, could also induce neurotoxic effects by activation of astrocytes and microglia, which causes neuroinflammation [84, 85].

Recently, a battery of tests, including hemolytic and oxidative stress markers, *in vitro* ROS generation and the comet assay, has been applied to evaluate cytotoxic and genotoxic effects of ZnO-NPs on human erythrocytes and lymphocytes [86]. The authors concluded that, similarly to dissolved Zn, ZnO-NPs concentrations above 50 ppm. are cytotoxic and genotoxic, due to the enhancement of oxidative stress induced by ROS generation. In addition to disruption of cellular Zn homeostasis, alteration of multiple enzymatic activities, and interaction with biomolecules, exacerbation of oxidative stress is the most recognized mechanism through which ZnO-NPs induce toxic effects. Accordingly, it has been demonstrated that the cytotoxic effect of ZnO-NPs is more pronounced in human cells previously exposed transiently to sublethal doses of H_2O_2, a standard oxidative stress-inducing agent [87]. One important scenario of the consequences of human exposure to ZnO-NPs was anticipated by the authors: individuals who suffer from diseases associated with increased oxidative stress (i.e., asthma, atherosclerosis, cardiovascular diseases, chronic obstructive pulmonary disease, and neurodegenerative diseases) should be considered at additional risk upon exposure to ZnO-NPs.

The pharmaceutical industry makes use of Zn(II) compounds ($ZnCl_2$, ZnO, zinc pyrithione, $Zn(CH_3CO_2)_2$) as active substances, lubricants, and emollients for a long time [88]. Usually, zinc-containing medicines are intended to topic application as wound healings, anti-infectious, disinfectants, and lubricants (**Table 3**). Apart from ZnO-NPs use in pharmaceutical formulations (i.e., drug delivery) and medicine (i.e., bioimaging), they are also present in a large number of consumer products (protective sunscreens, hair care formulations, cosmetics, supplements, food additives, etc.), as already mentioned above (Section 1). Recently, the European Council on Cosmetic Products (The European Commission (2016), Commission Regulation (EU) 2016/621 of 21 April 2016) restricted the use of ZnO-NPs in spray products because it could lead to exposure of the consumer's lungs to ZnO-NPs by inhalation, and encouraged only oral and dermal use of ZnO-NPs, up to a maximum concentration in ready

for use preparation of 25%, with the following characteristics: (i) purity ≥96%, with *wurtzite* crystalline structure and physical appearance as clusters that are rod-like, star-like, and/or isometric shapes, with impurities consisting only of carbon dioxide and water, while any other impurities are less than 1% in total, (ii) median diameter of the particle number size distribution D50 (50% of the number below this diameter) >30 nm and D1 (1% below this size) >20 nm; (iii) water solubility <50 mg L^{-1}; and (iv) uncoated or coated with triethoxycaprylylsilane, dimethicone, dimethoxy diphenylsilane triethoxycaprylylsilane crosspolymer, or octyl triethoxy silane.

Up to date information highlights not only the genotoxicity and cytotoxicity of ZnO-NPs due dissolution, ROS generation, immunomodulatory, and apoptotic responses, but also their selective cytotoxicity [82, 89–93]. In general, cytotoxicity is thought to be a collateral effect to avoid. To evaluate the specific risks and benefits of human exposure to ZnO-NPs, its size, shape, degradability, agglomeration/aggregation propensity, adsorption ability, specific surface area, and interfacial chemical and physical reactivity should be considered intrinsic properties, which likely influence their biopersistence, cellular interactions, and bioactivities when compared with microparticulate and dissolved Zn during intentional and unintentional human exposures. For instance, ZnO-NPs sized 20–25 nm appear to exhibit higher antibacterial and antifungal activity than other ZnO forms [15]. Sublethal concentrations of ZnO-NPs (surface area of 10.7 ± 0.7 nm) reduce the mitochondrial membrane potential, leading to a dose-dependent increase in gluconeogenesis and glycogenolysis, which could not be only attributed to dissolution of ZnO-NPs in extracellular fluids [94]. Kao et al. [85] proposed that ZnO-NPs are mainly internalized by endocytosis and dissolved in endosomes, raising the cytosolic free Zn^{2+} concentration, which is further sequestrated by mitochondria leading to cell apoptosis, due to mitochondrial dysfunction and caspases activation. ZnO-NPs readily dissolve in artificial lysosomal fluid (pH 4.5), but form aggregates and precipitates in the slight alkaline interstitial fluid [95].

Zinc compound	Route of administration	Role in formulation	Target organ	Clinical recommendations
Zinc acetate	Topic	Active ingredient: antibacterial action	Skin	Treatment of inflammatory acne, characterized by bacterial involvement
Zinc chloride	Topic	Active ingredient: antibacterial, analgesic, and healing actions	Mouth/ oropharynx	Treatment of gingivitis and stomatitis; relief of toothache; oral hygiene
Zinc oxide	Topic	Active ingredient: adjuvant of healing	Skin	Treatment of diaper dermatitis
	Topic	Emollient/lubricant: antihemorrhoidal action	Anus/lower rectum	Symptomatic treatment of hemorrhoids
	Topic	Emollient/lubricant: soothing, smoothing, and moisturizing actions	Skin	Symptomatic treatment of dry and scaly lesions, especially of ichthyosis, psoriasis, and eczema Disinfection and

Zinc compound	Route of administration	Role in formulation	Target organ	Clinical recommendations
				hygiene of the skin and mucous membranes, superficial wounds, and diaper dermatitis
Zinc pyrithione	Topic	Active ingredient: antibacterial and antifungal actions	Skin	Treatment of pityriasis versicolor, tinea pedis, psoriasis, seborrheic dermatitis, eczema, and vitiligo

Table 3. Commonly used medications that contain Zn for topical application.

In contract with small molecules that are translocated across the plasma membrane by passive diffusion or active transport, nanosized particles are internalized by cells mainly through endocytosis. This feature of NPs offers a myriad of opportunities for specific cellular targeting, controlled drug delivery, and bioimaging. The endocytotic capability varies significantly among cellular populations. Phagocytes (i.e., macrophages, monocytes, neutrophils, dendritic, and mast cells) are chemotaxing cells that move toward site of infections causing inflammation, play a central role in innate immunity response, and stimulate lymphocytes to produce antibodies (adaptive immunity) by antigen presentation. ZnO-NPs coating that favors interaction with opsonin, scavenger, or Toll-like receptors should enhance selective internalization by phagocytes. Uncoated ZnO-NPs should also be engulfed by lymphocytes, erythrocytes, fibroblasts, epithelial, and endothelial cells. Given that ZnO-NPs are microscopic particle with at least one dimension less than 100 nm, they can be internalized by any living cell by micro- and pinocytosis. The subcellular availability of ZnO-NPs should greatly depend on the specific endocytotic pathway (i.e., clathrin-dependent, caveolae-dependent, clathrin- and caveolae-independent, receptor-mediated) involved in the internalization process. For instance, ZnO-NPs loaded in caveolae vesicles may reach the endoplasmic reticulum and the nucleus, since caveosomes are pH neutral multivesicular bodies [96]. Conversely, internalization of ZnO-NPs through LDL receptor-mediated endocytosis should raise the cytosolic zinc ion significantly, due to dissolution of ZnO in the acidic lysosomal environment. Accordingly, it has been experimentally demonstrated by using ICP-MS and fluorescent-labeled ZnO dissolution occurs in endosomes, and that nondissolved ZnO-NPs enter caveolae in BEAS-2B cells (human bronchial epithelial cells) and enter lysosomes in RAW 264.7 cells (mouse leukemic monocyte macrophage cell) in which smaller particle remnants dissolve [90]. In the support of cell-specific behavior of stable aqueous solutions of monodispersed ZnO-NPs is the fact that ZnO-NPs doses exhibiting negligible cytotoxic effects to osteogenically differentiated mesenchymal stem cells were lethal to proliferating pluripotent mesenchymal stem cells [97]. As already mentioned above (Section 1), ZnO-NPs selectively induce apoptosis, mediated by reactive oxygen species via p53 pathway, in cancer cells (human hepatocellular carcinoma HepG2, human lung adenocarcinoma A549, and human bronchial epithelial BEAS-2B), but did not affect normal astrocytes and hepatocytes [98]. Thereby, to better explore therapeutic advantages and prevent unwanted cytotoxic effects and the potential of ZnNPs in terms of clinical diagnosis, it is important to perform a holistic analysis of the characteristics of ZnNPs,

the administration route, the zinc body burden, the target cells, and the relevant physiological processes in each specific case.

5. Conclusions, next steps, and opportunities

The ever increasing literature on ZnO-NPs clearly demonstrates the current value and applications of these particles and tremendous potential for future applications. There are a large number of challenges associated with its safe use, when compared with commonly tested substances. As for other nanoparticles, the advantages have to be carefully weight against potential pernicious effects. The currently available data clearly demonstrate the ability of ZnO-NPs to induce acute effects on fish, although at concentrations higher than those estimated to be present in the environment. Nonetheless, the long-term effects are yet to be explored. The considerable lack of information in terms of how these particles are released in the environment, at which levels, and in what form make the establishment of maximum allowed concentration a difficult task, based on available toxicity tests. More studies have to be conducted to explore the behavior of particles upon alterations of receiving media characteristics (e.g., ionic strength UV/radiation) and their fate. This information is essential for environmentally relevant ecotoxicological studies.

It is expected that, in the very near future, advances in analytical techniques allow quantification and accurate characterization of nanoparticles in environmental matrices which will allow the establishment of potentially impacted areas, monitoring of levels and effects on biota from those sites. Also, the need to the development of more effective wastewater treatments will potentially reduce the risk of the increased production of nanoparticle containing materials.

As can be seen from the literature, a broad range of applications of nanomaterials, in particular ZnO-NPs, exists on human activities. In this chapter, the benefits of ZnO nanomaterials are clearly recognized on a myriad of applications, having a great potential for the diagnosis, imaging, drug delivery, and treatment of several pathologies. Other areas within agricultural domain and energy resources have also relevant applications. Moreover, great potentials for their applications on aquaculture improving fish growth were documented.

The disposal and fate of ZnO-NPs into the environment may represent a risk to aquatic biota. This chapter highlights the significance in considering their fate and behavior into water bodies and its role on aquatic organisms, particularly fish. The published literature undoubtedly illustrates that ZnO-NPs have different toxic effects on microorganisms, rodents, human cells, and fish depending on their physicochemical features. In addition, the trophic transfer of these nanomaterials to humans through diet (i.e., by consuming contaminated fish) warrants special care. Therefore, disposal of ZnO-NPs deserves more attention since bioaccumulation of these elements may occur on aquatic species with impact on both human and environmental health. Precaution and more strict rules must be delimited for disposal of ZnO-NPs into the aquatic environments.

Zinc ion homeostasis is vital for humans and is closely linked with the homeostasis of other metal ions, particularly iron and copper. Nowadays, hypozincemia and hyperzincemia are two

pathophysiological conditions of which enduring prevalence is also related to malnutrition during aging and emerging lifestyle diseases (i.e., obesity) in industrialized countries. While the risks of using ZnO-NPs are not fully understood, the advantages of its emerging applications, including in the therapeutic and diagnostic areas, are already widely recognized. Probably, the toxicity of ZnO-NPs for man is not superior to the zinc ion itself, and nanoparticulate forms appear to enable interaction with specific cell cycle states (i.e., proliferating cells) and selective interference with important physiological processes, allowing not only selection of the administration route of ZnO-NPs but also the cellular internalization pathway and further intracellular distribution.

The balance of the positive aspects of these nanomaterials and risks caused in some aquatic species, particularly on fish, targeting possible implications for human health deserve a continuous monitoring. Although safety measures have been assumed during industrial production, storage, and removal of these nanomaterials, a constant monitoring of possible risks for aquatic life and ultimately humans is needed.

As a general conclusion, it is expected that in the near future, there is an increase in the use of ZnO-NPs for various purposes. Comprehensive understanding of their toxic effect is needed for their prolonged use.

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References

[1] Nohynek GJ, Lademann J, Ribaud C, Roberts MS. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. Critical Reviews in Toxicology. 2007;37:251–277. doi:10.1080/10408440601177780

[2] Song Z, Kelf TA, Sanchez WH, Roberts MS, Rička J, Frenz M, et al. Characterization of optical properties of ZnO nanoparticles for quantitative imaging of transdermal transport. Biomedical Optics Express. 2011;2:3321–3333. doi:10.1364/BOE.2.003321

[3] Dhobale S, Thite T, Laware SL, Rode CV, Koppikar SJ, Ghanekar R-K, et al. Zinc oxide nanoparticles as novel alpha-amylase inhibitors. Journal of Applied Physics. 2008;104:094907. doi:10.1063/1.3009317

[4] Rasmussen JW, Martinez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opinion on Drug Delivery. 2010;7:1063–1077. doi:10.1517/17425247.2010.502560

[5] Bisht G, Rayamajhi S. ZnO nanoparticles: a promising anticancer agent. Nanobiomedicine. 2016;9:1–11. doi:10.5772/63437

[6] Selvakumari DR, Mahalakshmi V, Subhashini P, Lakshminarayan N. Anti cancer activity of ZnO nanoparticles on MCF7 (breast cancer cell) and A549 (lung cancer cell). Asian Research Publishing Network Journal of Engineering and Applied Sciences. 2015;10:5418–5421.

[7] Wahab R, Kaushik NK, Kaushik N, Choi EH, Umar A, Dwivedi S, et al. ZnO nanoparticles induces cell death in malignant human T98G gliomas, KB and non-malignant HEK cells. Journal of Biomedical Nanotechnology. 2013;9:1181–1189. doi:10.1166/jbn.2013.1652

[8] Wahab R, Dwivedi S, Umar A, Singh S, Hwang IH, Shin H-S, et al. ZnO nanoparticles induce oxidative stress in Cloudman S91 melanoma cancer cells. Journal of Biomedical Nanotechnology. 2013;9:441–449. doi:10.1166/jbn.2013.1593

[9] Vinardell M, Mitjans M. Antitumor activities of metal oxide nanoparticles. Nanomaterials. 2015;5:1004–1021. doi:10.3390/nano5021004

[10] Namvar F, Rahman HS, Mohamad R, Azizi S, Tahir PM, Chartrand MS, et al. Cytotoxic effects of biosynthesized zinc oxide nanoparticles on murine cell lines. Evidence-Based Complementary and Alternative Medicine. 2015;2015:11. doi: 10.1155/2015/593014

[11] Shen W, Xiong H, Xu Y, Cai S, Lu H, Yang P. ZnO–poly(methyl methacrylate) nanobeads for enriching and desalting low-abundant proteins followed by directly MALDI-TOF MS analysis. Analytical Chemistry. 2008;80:6758–6763. doi: 10.1021/ac801001b

[12] Yamaki K, Yoshino S. Comparison of inhibitory activities of zinc oxide ultrafine and fine particulates on IgE-induced mast cell activation. BioMetals. 2009;22:1031–1040. doi: 10.1007/s10534-009-9254-z

[13] Augustine R, Dominic EA, Reju I, Kaimal B, Kalarikkal N, Thomas S. Investigation of angiogenesis and its mechanism using zinc oxide nanoparticle-loaded electrospun tissue engineering scaffolds. RSC Advances. 2014;4:51528–51536. doi:10.1039/C4RA07361D

[14] Jiang J, Oberdörster G, Biswas P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. Journal of Nanoparticle Research. 2009;11:77–89. doi:10.1007/s11051-008-9446-4

[15] Navale G, Thripuranthaka M, Late D, Shinde S. Antimicrobial activity of ZnO nanoparticles against pathogenic bacteria and fungi. JSM Nanotechnology Nanomedicine. 2015;3:1033. doi:10.3390/na7042833

[16] Rashmirekha P, Kumar MR, Soumitra M, Avinash P, Mitali S, Baskarlingam V, et al. Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. Nanomedicine: Nanotechnology, Biology and Medicine. 2014;10:1195–1208. doi:10.1016/j.nano.2014.02.012

[17] Mishra YK, Adelung R, Röhl C, Shukla D, Spors F, Tiwari V. Virostatic potential of micro-nano filopodia-like ZnO structures against herpes simplex virus-1. Antiviral Research. 2011;92:305–312. doi:10.1016/j.antiviral.2011.08.017

[18] Antoine TE, Mishra YK, Trigilio J, Tiwari V, Adelung R, Shukla D. Prophylactic, therapeutic and neutralizing effects of zinc oxide tetrapod structures against herpes simplex virus type-2 infection. Antiviral Research. 2012;96:363–375. doi:10.1016/j.antiviral.2012.09.020

[19] Narayanan PM, Wilson WS, Abraham AT, Sevanan M. Synthesis, characterization, and antimicrobial activity of zinc oxide nanoparticles against human pathogens. BioNanoScience. 2012;2:329–335. doi:10.1007/s12668-012-0061-6

[20] Degen A, Kosec M. Effect of pH and impurities on the surface charge of zinc oxide in aqueous solution. Journal of the European Ceramic Society. 2000;20:667–673. doi: 10.1016/S0955-2219(99)00203-4

[21] Prasad TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, et al. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of Plant Nutrition. 2012;35:905–927. doi: 10.1080/01904167.2012.663443

[22] Lin D, Xing B. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environmental Pollution. 2007;150:243–250. doi:10.1016/j.envpol.2007.01.016

[23] Ge Y, Schimel JP, Holden PA. Identification of soil bacteria susceptible to TiO_2 and ZnO nanoparticles. Applied and Environmental Microbiology. 2012;78:6749–6758. doi: 10.1128/aem.00941-12

[24] Pereira AS, Trindade T. Chapter 9 Nano dimensional ZnO: new chemical insights from an old material. PJ Thomas, P O´Brien (Eds.) Nanoscience, vol. 2. Royal Society of Chemistry; London, 2014. p. 255–285. doi:10.1039/9781849737623-00255

[25] Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, et al. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Letters. 2015;7:219–242. doi:10.1007/s40820-015-0040-x

[26] Kołodziejczak-Radzimska A, Jesionowski T. Zinc oxide—from synthesis to application: a review materials. 2014;7:2833. doi:10.3390/ma7042833

[27] Bala N, Saha S, Chakraborty M, Maiti M, Das S, Basu R, et al. Green synthesis of zinc oxide nanoparticles using Hibiscus subdariffa leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. RSC Advances. 2015;5:4993–5003. doi:10.1039/C4RA12784F

[28] Sangeetha G, Rajeshwari S, Venckatesh R. Green synthesis of zinc oxide nanoparticles by Aloe barbadensis miller leaf extract: structure and optical properties. Materials Research Bulletin. 2011;46:2560–2566. doi:10.1016/j.materresbull.2011.07.046

[29] Aneesh PM, Vanaja KA, Jayaraj MK. Synthesis of ZnO nanoparticles by hydrother mal method. Nanophotonic Materials. 2007. p. 66390J-1–66399J-9. doi: 10.1117/12.730364

[30] Ramimoghadam D, Hussein MZB, Taufiq-Yap YH. Synthesis and characterization of ZnO nanostructures using palm olein as biotemplate. Chemistry Central Journal. 2013;7:1–10. doi:10.1186/1752-153x-7-71

[31] Soni J, Koser A. Synthesis of zinc oxide nanoparticles using different concentration of capping agent. International Journal of Technology Innovations and Research. 2015;16:2321–1814.

[32] Wang Y, Zhang C, Bi S, Luo G. Preparation of ZnO nanoparticles using the direct precipitation method in a membrane dispersion micro-structured reactor. Powder Technology. 2010;202:130–136. doi:10.1016/j.powtec.2010.04.027

[33] Giri PK, Bhattacharyya S, Chetia B, Kumari S, Singh DK, Iyer PK. High-yield chemical synthesis of hexagonal ZnO nanoparticles and nanorods with excellent optical properties. Journal of Nanoscience and Nanotechnology. 2012;12:201–206. doi:10.1166/jnn.2012.5113

[34] Oudhia A, Kullkarni P, Sarma S. Green synthesis of zinc oxide nano tubes for bio application. International Journal of Advanced Engineering Research and Studies. 2015;4:280–281.

[35] Barreto GP, Morales G, Quintanilla MLL. Microwave assisted synthesis of ZnO nanoparticles: effect of precursor reagents, temperature, irradiation time, and additives on nano-ZnO morphology development. Journal of Materials. 2013;2013:11. doi:10.1155/2013/478681

[36] Kumar H, Rani R. Structural and optical characterization of zinc oxide nanoparticles synthesized by micro emulsion. International Letters of Chemistry, Physics, and Astronomy. 2013;14:26–36.

[37] Tsuzuki T, McCormick PG. ZnO nanoparticles synthesised by mechanochemical processing. Scripta Materialia. 2001;44:1731–1734.

[38] Bystrzejewska-Piotrowska G, Golimowski J, Urban PL. Nanoparticles: their potential toxicity, waste and environmental management. Waste Management. 2009;29:2587–2595. doi:10.1016/j.wasman.2009.04.001

[39] Sun TY, Gottschalk F, Hungerbühler K, Nowack B. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. Environmental Pollution. 2014;185:69–76. doi:10.1016/j.envpol.2013.10.004

[40] Batley GE, Kirby JK, McLaughlin MJ. Fate and risks of nanomaterials in aquatic and terrestrial environments. Accounts of Chemical Research. 2013;46:854–862. doi:10.1021/ar2003368

[41] Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, et al. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environmental Toxicology and Chemistry. 2008;27:1825–1851. doi:10.1897/08-090.1

[42] Maurer-Jones MA, Gunsolus IL, Murphy CJ, Haynes CL. Toxicity of engineered nanoparticles in the environment. Analytical Chemistry. 2013;85:3036–3049. doi:10.1021/ac303636s

[43] Barreto Â, Luis LG, Girão AV, Trindade T, Soares AMVM, Oliveira M. Behavior of colloidal gold nanoparticles in different ionic strength media. Journal of Nanoparticle Research. 2015;17:1–13. doi:10.1007/s11051-015-3302-0

[44] Yung MMN, Mouneyrac C, Leung KMY. Ecotoxicity of zinc oxide nanoparticles in the marine environment. In: Bhushan B, editor. Encyclopedia of Nanotechnology. Dordrecht: Springer Netherlands; 2014. p. 1–17. doi: 10.1007/978-94-007-6178-0_100970-1

[45] Liu L, Wang X, Yang X, Fan W, Wang X, Wang N, et al. Preparation, characterization, and biotoxicity of nanosized doped ZnO photocatalyst. International Journal of Photoenergy. 2014;2014:8. doi:10.1155/2014/475825

[46] Akhil K, Chandran P, Sudheer Khan S. Influence of humic acid on the stability and bacterial toxicity of zinc oxide nanoparticles in water. Journal of Photochemistry and Photobiology B: Biology. 2015;153:289–295. doi:10.1016/j.jphotobiol.2015.10.007

[47] Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS. Comparative toxicity of nanoparticulate ZnO, Bulk ZnO, and $ZnCl_2$ to a freshwater microalga (Pseudokirchneriella subcapitata): the importance of particle solubility. Environmental Science and Technology. 2007;41:8484–8490. doi:10.1021/es071445r

[48] Bian S-W, Mudunkotuwa IA, Rupasinghe T, Grassian VH. Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: influence of pH, ionic strength, size, and adsorption of humic acid. Langmuir. 2011;27:6059–6068. doi:10.1021/la200570n

[49] Som C, Nowack B, Krug HF, Wick P. Toward the development of decision supporting tools that can be used for safe production and use of nanomaterials. Accounts of Chemical Research. 2013;46:863–872. doi:10.1021/ar3000458

[50] Adam N, Schmitt C, De Bruyn L, Knapen D, Blust R. Aquatic acute species sensitivity distributions of ZnO and CuO nanoparticles. Science of the Total Environment. 2015;526:233–242. doi:10.1016/j.scitotenv.2015.04.064

[51] Zhao X, Wang S, Wu Y, You H, Lv L. Acute ZnO nanoparticles exposure induces developmental toxicity, oxidative stress and DNA damage in embryo-larval zebrafish. Aquatic Toxicology. 2013;136–137:49–59. doi:10.1016/j.aquatox.2013.03.019

[52] Callaghan NI, Allen GJP, Robart TE, Dieni CA, MacCormack TJ. Zinc oxide nanoparticles trigger cardiorespiratory stress and reduce aerobic scope in the white sucker, Catostomus commersonii. NanoImpact. 2016;2:29–37. doi:10.1016/j.impact.2016.06.004

[53] Dieni CA, Callaghan NI, Gormley PT, Butler KMA, MacCormack TJ. Physiological hepatic response to zinc oxide nanoparticle exposure in the white sucker, Catostomus commersonii. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology. 2014;162:51–61. doi:10.1016/j.cbpc.2014.03.009

[54] Wong SWY, Leung PTY, Djurišić AB, Leung KMY. Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility.

Analytical and Bioanalytical Chemistry. 2010;396:609–618. doi:10.1007/s00216-009-3249-z

[55] Hao L, Chen L, Hao J, Zhong N. Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (Cyprinus carpio): a comparative study with its bulk counterparts. Ecotoxicology and Environmental Safety. 2013;91:52–60. doi:10.1016/j.ecoenv.2013.01.007

[56] Fan W, Li Q, Yang X, Zhang L. Zn subcellular distribution in liver of goldfish (Carassius auratus) with exposure to zinc oxide nanoparticles and mechanism of hepatic detoxification. PLoS One. 2013;8:e78123. doi:10.1371/journal.pone.0078123

[57] Tomilina II, Gremyachikh VA, Grebenyuk LP, Klevleeva TR. The effect of zinc oxide nano- and microparticles and zinc ions on freshwater organisms of different trophic levels. Inland Water Biology. 2014;7:88–96. doi:10.1134/s1995082914010155

[58] Faiz H, Zuberi A, Nazir S, Rauf M, Younus N. Zinc oxide, zinc sulfate and zinc oxide nanoparticles as source of dietary zinc: comparative effects on growth and hematological indices of juvenile grass carp (Ctenopharyngodon idella). International Journal of Agriculture and Biology. 2015;17:568–574. doi:10.17957IJAB/17.3.14.446

[59] Suganthi MM, Sadiq BA, Syed MH, Basu H, Singhal R. Zinc oxide (ZnO) nanoparticles induced histopathological changes in the liver of freshwater fish, Cyprinus carpio. Cibtech Journal of Zoology. 2014;3:74–77.

[60] Subashkumar S, Selvanayagam M. Zinc oxide (ZnO) nanoparticles induced histopathological changes in the liver of freshwater fish, Cyprinus carpio. Cibtech Journal of Zoology. 2014;3:74–77.

[61] Subashkumar S, Selvanayagam M. First report on: acute toxicity and gill histopathology of fresh water fish Cyprinus carpio exposed to Zinc oxide (ZnO) nanoparticles. International Journal of Scientific and Research Publications. 2014;4:1–4.

[62] Skjolding LM, Winther-Nielsen M, Baun A. Trophic transfer of differently functionalized zinc oxide nanoparticles from crustaceans (Daphnia magna) to zebrafish (Danio rerio). Aquatic Toxicology. 2014;157:101–108. doi:10.1016/j.aquatox.2014.10.005

[63] AL-Taee SKI, AL-Hamdani AHA. Pathological study of lethal concentration of n-ZnO in common carp Cyprinus carpio L. Basrah Journal of Veterinary Research. 2013;12:200–207.

[64] Nowack B, Mueller NC, Krug HF, Wick P. How to consider engineered nanomaterials in major accident regulations? Environmental Sciences Europe. 2014;26:1–10. doi:10.1186/2190-4715-26-2

[65] Brown JJL. Zinc fume fever. The British Journal of Radiology. 1988;61:327–329. doi:10.1259/0007-1285-61-724-327

[66] Rohrs LC. Metal-fume fever from inhaling zinc oxide. AMA Archives of Internal Medicine. 1957;100:44–49. doi:10.1001/archinte.1957.00260070058005

[67] Zaichick VY, Sviridova TV, Zaichick SV. Zinc in the human prostate gland: normal, hyperplastic and cancerous. International Urology and Nephrology. 1997;29:565–574. doi:10.1007/bf02552202

[68] Smith JC, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clinical Chemistry. 1979;25:1487–1491.

[69] Colvin RA, Holmes WR, Fontaine CP, Maret W. Cytosolic zinc buffering and muffling: their role in intracellular zinc homeostasis. Metallomics. 2010;2:306–317. doi:10.1039/B926662C

[70] Kambe T, Tsuji T, Hashimoto A, Itsumura N. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiological Reviews. 2015;95:749–784. doi:10.1152/physrev.00035.2014

[71] Andreini C, Banci L, Bertini I, Rosato A. Counting the zinc-proteins encoded in the human genome. Journal of Proteome Research. 2006;5:196–201. doi: 10.1021/pr050361j

[72] Gamsjaeger R, Liew CK, Loughlin FE, Crossley M, Mackay JP. Sticky fingers: zinc-fingers as protein-recognition motifs. Trends in Biochemical Sciences. 2007;32:63–70. doi:10.1016/j.tibs.2006.12.007

[73] Beyersmann D, Haase H. Functions of zinc in signaling, proliferation and differentiation of mammalian cells. Biometals. 2001;14:331–341. doi:10.1023/a:1012905406548

[74] Maret W. Zinc biochemistry: from a single zinc enzyme to a key element of life. Advances in Nutrition: An International Review Journal. 2013;4:82–91. doi:10.3945/an.112.003038

[75] Kim AM, Bernhardt ML, Kong BY, Ahn RW, Vogt S, Woodruff TK, et al. Zinc sparks are triggered by fertilization and facilitate cell cycle resumption in mammalian eggs. ACS Chemical Biology. 2011;6:716–723. doi:10.1021/cb200084y

[76] Frederickson CJ, Koh J-Y, Bush AI. The neurobiology of zinc in health and disease. Nature Reviews on Neuroscience. 2005;6:449–462.

[77] Brown KH, Wuehler SE, Peerson JM. The Importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food and Nutrition Bulletin. 2001;22:113–125. doi:10.1177/156482650102200201

[78] Fosmire GJ. Zinc toxicity. The American Journal of Clinical Nutrition. 1990;51:225–227.

[79] King JC, Shames DM, Woodhouse LR. Zinc homeostasis in humans. The Journal of Nutrition. 2000;130:1360S–1366S.

[80] Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. Journal of Trace Elements in Medicine and Biology. 2006;20:3–18. doi: 10.1016/j.jtemb.2006.01.006

[81] Lin W, Xu Y, Huang C-C, Ma Y, Shannon KB, Chen D-R, et al. Toxicity of nano- and micro-sized ZnO particles in human lung epithelial cells. Journal of Nanoparticle Research. 2009;11:25–39. doi:10.1007/s11051-008-9419-7

[82] Saptarshi SR, Duschl A, Lopata AL. Biological reactivity of zinc oxide nanoparticles with mammalian test systems: an overview. Nanomedicine. 2015;10:2075–2092. doi: 10.2217/nnm.15.44

[83] Thompson EA, Sayers BC, Glista-Baker EE, Shipkowski KA, Taylor AJ, Bonner JC. Innate immune responses to nanoparticle exposure in the lung. Journal of Environmental Immunology and Toxicology. 2014;1:150–156. doi:10.7178/jeit.23

[84] Simkó M, Mattsson M-O. Risks from accidental exposures to engineered nanoparticles and neurological health effects: a critical review. Particle and Fibre Toxicology. 2010;7:1–15. doi:10.1186/1743-8977-7-42

[85] Kao Y-Y, Cheng T-J, Yang D-M, Wang C-T, Chiung Y-M, Liu P-S. Demonstration of an olfactory bulb–brain translocation pathway for ZnO nanoparticles in rodent cells in vitro and in vivo. Journal of Molecular Neuroscience. 2012;48:464–471. doi:10.1007/s12031-012-9756-y

[86] Khan M, Naqvi AH, Ahmad M. Comparative study of the cytotoxic and genotoxic potentials of zinc oxide and titanium dioxide nanoparticles. Toxicology Reports. 2015;2:765–774. doi:10.1016/j.toxrep.2015.02.004

[87] Heng BC, Zhao X, Xiong S, Woei Ng K, Yin-Chiang Boey F, Say-Chye Loo J. Toxicity of zinc oxide (ZnO) nanoparticles on human bronchial epithelial cells (BEAS-2B) is accentuated by oxidative stress. Food and Chemical Toxicology. 2010;48:1762–1766. doi: 10.1016/j.fct.2010.04.023

[88] Gupta M, Mahajan VK, Mehta KS, Chauhan PS. Zinc therapy in dermatology: a review. Dermatology Research and Practice. 2014;2014:11. doi:10.1155/2014/709152

[89] Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. Science. 2006;311:622–627. doi:10.1126/science.1114397

[90] Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, et al. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano. 2008;2:2121–2134. doi:10.1021/nn800511k

[91] Shvedova AA, Kagan VE, Fadeel B. Close encounters of the small kind: adverse effects of man-made materials interfacing with the nano-cosmos of biological systems. Annual Review of Pharmacology and Toxicology. 2010;50:63–88. doi:10.1146/annurev.pharmtox.010909.105819

[92] Win-Shwe T-T, Fujimaki H. Nanoparticles and neurotoxicity. International Journal of Molecular Sciences. 2011;12:6267–6280. doi:10.3390/ijms12096267

[93] Kou L, Sun J, Zhai Y, He Z. The endocytosis and intracellular fate of nanomedicines: implication for rational design. Asian Journal of Pharmaceutical Sciences. 2013;8:1–10. doi:10.1016/j.ajps.2013.07.001

[94] Filippi C, Pryde A, Cowan P, Lee T, Hayes P, Donaldson K, et al. Toxicology of ZnO and TiO_2 nanoparticles on hepatocytes: impact on metabolism and bioenergetics. Nanotoxicology. 2015;9:126–134. doi:10.3109/17435390.2014.895437

[95] Adamcakova-Dodd A, Stebounova LV, Kim JS, Vorrink SU, Ault AP, O'Shaughnessy PT, et al. Toxicity assessment of zinc oxide nanoparticles using sub-acute and sub-chronic murine inhalation models. Particle and Fibre Toxicology. 2014;11:1–15. doi: 10.1186/1743-8977-11-15

[96] Parton RG, Simons K. The multiple faces of caveolae. Nature Reviews on Molecular and Cell Biology. 2007;8:185–194. doi:10.1038/nrm2122

[97] Taccola L, Raffa V, Riggio C, Vittorio O, Iorio MC, Vanacore R, et al. Zinc oxide nanoparticles as selective killers of proliferating cells. International Journal of Nanomedicine. 2011;6:1129–1140. doi:10.2147/IJN.S16581

[98] Akhtar MJ, Ahamed M, Kumar S, Khan MAM, Ahmad J, Alrokayan SA. Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. International Journal of Nanomedicine. 2012;7:845–857. doi:10.2147/IJN.S29129

Chapter 6

Microplastics in Aquatic Environments and Their Toxicological Implications for Fish

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Additional information is available at the end of the chapter

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Abstract

The intensive use of plastics and derivatives during the last century has increased the contamination of animal habitats. The breakdown of these primary plastics in the environment results in microplastics (MP), small fragments of plastic typically <1–5 mm in size. Apart from the potential negative effects of the MPs *per se*, it is generally assumed that microplastics may increase the exposure of marine aquatic organisms to chemicals associated with the plastics. In addition, to enhance the performance of plastics, additives are added during manufacture. Furthermore, they are active in absorbing other contaminants and be used as vectors of highly and well-documented persistent contaminants. Finally, these small MPs are easily ingested by animals and affect their physiology and behaviour. Thus, aquatic living organisms are continuously exposed to these MPs, and associated contaminants, and could suffer from its contamination but also introduce them into the food chain.

Keywords: Microplastics, toxicants, aquatic environment, fish

1. Introduction

The production of synthetic polymers has increased more than 100-fold since the middle of the twentieth century to reach the 280 million tonnes of plastics produced annually worldwide most of which is destined for disposable use [1]. High production coupled with the physical characteristics of most plastics, such as their chemical inertness and very slow biodegradation rates, results in an accumulation of plastic debris in the environment [2]. Routes of discharge such as improper waste disposal, insufficient waste management and urban run-offs [3] may

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lead to significant amounts of these plastics entering the aquatic environment [4, 5]. It is a long-recognized fact that marine plastic debris contaminates the oceans and seas of all the world [3, 6, 7]. In the marine environment, plastics undergo a process of weathering and fragmentation that breaks down macrodebris into smaller micro- and nanodebris. This fragmentation of plastic is caused by a combination of mechanical forces, for example waves and/or photochemical processes triggered by sunlight. Some 'degradable' plastics are even designed to fragment quickly into small particles, although the resulting material does not necessarily biodegrade [8].

The terms 'microplastics' (MP) and 'microlitter' have been defined differently by various researchers. Gregory and Andrady [9] defined microlitter as the barely visible particles that pass through a 500-μm sieve but are retained by a 67-μm sieve (≈0.06–0.5 mm in diameter), while particles larger than this were called mesolitter. Others [10–12] defined the MPs as being in the size range <5 mm (recognizing 333 μm as a practical lower limit when neuston nets are used for sampling). Microplastic particles may further fragment into 'nanoplastics', a term that has not been defined uniformly in the literature, and may refer to <100-μm particles of plastic [13].

Microplastics have been accumulating in the environment for nearly half a century and are found in oceans worldwide [3] including in the Antarctic [7]. Despite this worldwide dissemination of plastic fragments, the global load of plastics on the open ocean surface has been estimated to be far less than might be expected, but nevertheless increasing. Thus, the potential effects of microplastics on marine ecosystems are still far from being well understood [14]. It is believed that the virging MPs are not chemical contaminants to marine organism, but they can produce physical problems such as digestive congestion. However, they can be loaded with many substances to fit the virgin MPs to industry and consumer demand (e.g. additives, preservatives, etc.). In addition, these MPs can also adsorb contaminants present in the environment and act as vectors. Therefore, in this chapter, we shall summarize some important aspects of the microplastics found in the marine environments and some of the effects described in fish biota.

2. Chemical nature

Plastics are usually synthesized from fossil fuels, but biomass can also be used as feedstock. The most commonly used plastic materials, the also called virgin plastics, are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET), which, together, represent approximately 90% of total world plastic production [15]. They are elements of high molecular weight and are non-biodegradable and therefore extremely persistent in the environment. PE, PP, PVC, PS, PET and polyurethane (PUR) are widely used resins (29, 19, 12, 8, 6, and 7% of global production, respectively) [16]. Plastics present many advantages since they are inexpensive, water- and corrosion-resistant, chemically inert, easily moulded and exhibit good thermal and electrical insulating properties. However, plastics also present many disadvantages, being non-renewable resources and

sources of contamination by additive compounds; they suffer embrittlement at low temperatures and deformation under loads; they need costly recycling processes and are highly resistant to degradation, etc. The behaviour of plastics in the environment will differ according to their chemical nature and physical properties. A reflection of this is the description of microplastics found in marine environments in different studies (**Table 1**).

Polymer type	**% Studies (*n*)**
Polyethylene (PE)	31 (33)
Polypropylene (PP)	25 (27)
Polystyrene (PS)	16 (17)
Polyamide (nylon) (PA)	6.0 (7)
Polyester (PES)	3.7 (4)
Acrylic (AC)	3.7 (4)
Polyoxymethylene (POM)	3.7 (4)
Polyvinyl alcohol (PVA)	2.8 (3)
Polyvinyl chloride (PVC)	1.8 (2)
Poly methylacrylate (PMA)	1.8 (2)
Polyethylene terephthalate (PET)	0.9 (1)
Alkyd (AKD)	0.9 (1)
Polyurethane (PU)	0.9 (1)

Table 1. Frequency of occurrence of different polymer types in microplastic debris sampled at sea or in marine sediments [17].

3. Sources

Microplastics comprise a very heterogeneous assemblage of particles that vary in size, shape, colour, chemical composition, density, and other characteristics. They can be subdivided according to their usage and source into (i) 'primary' MPs, produced either for indirect use as precursors (nurdles or virgin resin pellets), for the production of polymer consumer products or for direct use, for example in cosmetics, scrubs and abrasives and (ii) 'secondary' MPs, which result from the breakdown of larger plastic material into smaller fragments [18].

3.1. Primary: common consumer products

Microplastics (e.g. PE spheres) are used in personal care products such as toothpaste, facial and exfoliating creams, even though many consumers are not aware of this. In some cases, these MPs have replaced natural materials, such as seeds, shells or ground pumice ingredients. Usually, they are not filtered during wastewater treatment and are usually released directly

into the sea or other water bodies such as lakes and rivers. Microplastics are also found in synthetic textiles: wastewaters from washing synthetic clothes, such as shirts, contain more than 100 fibres per litre of water. According to a study by Browne et al. [19], on average, about 1900 MP fibres can be released in a single machine wash. Similar fibres have been observed in wastewater effluent and sludge near large urban centres.

3.2. Primary: industrial sources of microplastics

Plastic pellets are the raw material of plastic products. They are typically spherical or cylindrical in shape and millimetres in diameter. In addition, pellets are used in various industrial applications, including as ingredients of printing inks, paints spray, injection mouldings and abrasives [20]. A proportion of MPs used in these industrial applications enters the environment. The improvement in the management of operations in which plastic pellets are used could be a clear way to prevent them from entering the environment.

3.3. Secondary: plastic waste as a source of microplastics

Secondary microplastics are formed when larger plastic items are broken down. The rate at which fragmentation occurs is highly dependent on environmental conditions, especially temperature and the amount of UV light available [20]. Plastic debris can enter the ocean directly or can reach it through other water bodies or the atmosphere. The key to stopping plastic 'ocean trash' is to prevent such waste from entering the environment in the first place. Obviously, larger objects are easier to identify and control than smaller objects. About half of the world's population lives within 100 km of the coast, with an increasing population in that area. It is therefore highly likely that the amount of plastic waste entering the ocean from land-based sources will increase if significant changes are not made in the waste management on land.

4. Ecosystem distribution

The accuracy of MP emission estimates is currently hindered by lack of data. More specifically, information on MP transport efficiency in run-off and streams is missing. This is despite the large number of qualitative studies on microplastics in rivers and sediments [21–24]. Similarly, only limited assessments of MPs from sewage and canalizations, their retention by wastewater treatment plants and release by effluents are available [25, 26].

One of the most important factors affecting microplastic distribution in marine waters is the density of the materials (**Table 2**). Materials whose specific density is less than that of marine water (~1.02) may be located on the surface, while materials with a specific density greater than that of marine water may be sink (**Table 2**). Thus, being buoyant in water, PE and PP float in seawater and mainly affect ocean surfaces and deposits ashore [27, 28], while PVC, which is denser than seawater, affects the seabed, often next to the source [27].

Categories	Common applications	Specific density*
Polyethylene (PE)	Plastic bags, six-pack rings	0.91–0.94
Polypropylene (PP)	Rope, bottle caps, netting	0.90–0.92
Foamed polystyrene (PS)	Cups, buoy	0.01–1.05
Polystyrene (PS)	Tools, packaging	1.04–1.09
Polyvinyl chloride (PVC)	Bags, tubes	1.16–1.30
Polyamide or nylon	Rope	1.13–1.15
Polyethylene terephthalate (PET)	Bottles	1.34–1.39
Polyester resin+fibreglass	Textiles	>1.35
Polycarbonate (PC)	Electronic compounds	1.20–1.22
Cellulose acetate	Filter cigarettes	1.22–1.24
Polytetrafluoroethylene	Teflon, tubes	2.1–2.3

*Specific density expressed in g/cm^3. The density of plastics may change depending on additives and environmental processes. Based on References [5, 29, 30].

Table 2. Common applications and specific density of some plastics found in the marine environment.

Location	Microplastic concentrations	References
Pacific Ocean	27,000–448,000 particles per km^2	[33, 34]
	370,000 particles per km^2	[35]
	0.004–9200 particles per m^3	[36, 37]
Atlantic Ocean	2.5 particles per m^3	[38]
Indian Ocean	81.43 mg per kg*	[39]
Mediterranean Sea	0.16 particles per m^2	[40]
	0.62 particles per m^3	[41]

*Sediment samples.

Table 3. Microplastic concentrations observed in oceans of the world.

Moreover, the colonization of MPs by microalgae and other microorganisms increases plastic density, which has been shown to affect the vertical transport of MPs in an aquatic environment and their long-term distribution [31]. However, the chemical composition, particularly as a result of low amounts of additives, may partially explain the changes in microbiological colonization from one type of polymer to another. Also, for the same type of polymer, the chemical composition can vary considerably depending on chemical additives and the time passed in the environment. Hence, the distribution of MPs in the ecosystems may change according to these parameters, too. Long et al. [32] recently showed that MPs could be incorporated in microalgal homo-aggregates, demonstrating the existence of a pathway of vertical transport of MP from the surface layer to the floor of the ocean.

In addition, MP concentrations and/or quantities differ between sampling sites (**Table 3**). A significant variation between the microplastics sampled in different oceans is evident, but there are also differences between the areas of the same ocean or sea. Published works have detected different concentrations of MPs depending on the proximity to populated and/or contaminated areas.

5. Absorption of toxicants

To enhance the performance of plastics, additives are added during manufacture, such as reinforcing fibres, fillers, coupling agents, plasticizers, colorants, stabilizers (halogen stabilizers, antioxidants, ultraviolet absorbers and biological preservatives), adsorbed chemicals, and unreacted starting materials (monomers), processing aids (lubricants and flow control), flame retardants, peroxide, antistatic agent, and plasticizers [16, 42], which may leach out under conditions of use and accumulate in the environment [43]. Apart from the potential negative effects of the MPs *per se*, it is generally assumed that microplastics may increase the exposure of marine aquatic organisms to chemicals associated with the plastics, such as persistent organic pollutants (POPs) or plastic additives [44–47]. Thus, analytical study of marine MPs has revealed the composition of many toxicants adsorbed to them.

Additive	CAS	Log K_{OW}	Water solubility (mg/L)
UV stabilizers			
Benzophenone	119-61-9	3.18	None
Benzotriazol	95-14-7	1.44	1.98×10^4
Antioxidants			
Irganox 1024	32687-78-8	7.79	<1
Irganox 1098	23128-74-7	–	0.1
Irganox 1076	2082-79-3	<6	<0.01
Irganox 1010	6683-19-8	≈23	<0.01
Irganox 168	31570-04-4	>6	<0.005
Plasticisers			
Dimethyl phthalate	131-11-3	1.61	4.2×10^4
Diethyl phthalate	84-66-2	2.38	1.1×10^4
Di-n-butyl phthalate	84-74-2	4.45	112
Butylbenzyl phthalate	85-68-7	4.59	2.7
Bis(2-ethylhexyl) phthalate	17-81-7	7.5	0.003
Di-n-octyl phthalate	3-1307	8.06	0.02
Lubricants			

Additive	CAS	Log K_{OW}	Water solubility (mg/L)
n-Hexadecanoic acid	57-10-3	7.17	0.04
Oleic acid	112-80-1	7.64	None
Glycerol tricaprylate	538-23-8	9.20	0.40 (37°C)
Isopropyl myristate	110-27-0	7.17	2.44×10^{-2}
1-Eicosanol	629-96-9	8.70	1.5×10^{-3}
2-Hexyl-1-decanol	2425-77-6	6.66	0.1727
Octadecanamide	124-26-5	7.292	None
4-Methyl-benzenesulfonamide	70-55-3	0.82	3.16×10^{3}
Hexacosanol	506-52-5	11.65	1.438×10^{6}
Decanedioic acid, bis(2-ethylhexyl)	122-62-3	9.63	None
Fuel			
Pentadactyl ester trichloroacetic acid	74339-53-0	–	–
1,10-[2-methyl-2-(phenylthio)cyclopropenylidene] bisbenzene	56728-02-0	–	–
2,4-dimethyl-4-octanol	568123	3.51	188.9
Hexadecyl ester trichloroacetic acid	74339-54-1	9.1	6.223×10^{-5}
Intermediates			
HEHA	59130-69-7	11.15	4.127×10^{6}
2,3-Dihydroxypropyl ester hexadecanoic acid	542-44-9	4.364	None
Hexadecanoic acid ethyl ester	628-97-7	7.74	3.71×10^{3}
Behenic alcohol	661-19-8	9.68	1.5×10^{5}
Nonanoic acid	112-05-0	3.42	284
Pimaric acid	127-27-5	6.60	9.232×10^{2}
3,5-Di-tert-butyl-4-hydroxy phenyl propionic acid	20170-32-5	4.48	12.93
Abietic acid	514-10-3	6.51	8.96×10^{2}
Dehydroabietic acid	1740-19-8	6.35	8.161×10^{2}
Monomers and oligomers			
Bisphenol A	80-05-7	3.32	300
4-Hydroxyacetophenone	99-93-4	1.42	2.32×10^{4}
4-Hydroxyacetophenone	99-96-7	1.58	5×10^{3}
Flame retards			
PCBs	1336-36-3	3.76–8.26	$2.7–1.5 \times 10^{4}$
PBBs	67774-32-7	6.5–9.4	–
PBDE		5.52-11.22	5.6×10^{-10}–0.13
-tetraBDE	40088-47-9	5.87–6.16	1.1×10^{-2}

Additive	CAS	Log K_{OW}	Water solubility (mg/L)
-pentaBDE	32534-81-9	6.57	13.3×10^{-3}
-hexaBDE	36483-60-0	6.86–7.92	4.2×10^{-6}
-heptaBDE	68928-80-3	9.44	2.2×10^{-7}
-octaBDE	32536-52-0	6.29	5×10^{-4}
-nonaBDE	63936-56-1	11.22	5.6×10^{-10}
-decaBDE	1163-19-5	6.265	–
α-HBCD	134237-50-6	5.07	48.8
β-HBCD	134237-51-7	5.12	14.7
γ-HBCD	134237-52-8	5.47	2.1
TBBP-A	79-94-7	4.5	720
BTBPE	37853-59-1	7.88	19
DBDPE	84852-53-9	11.1	21
Anti-DP syn-DP	13560-89-9	9.3	250
Others			
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	82304-66-3	3.59	15.5
Glycerol 1-palmitate	32899-41-5	6.17	0.1252
(Z)-13-docosenamide	112-84-5	5.3	0.2
Di-tert-dodecyl disulfide	27458-90-8	6.1	None
1-Hexadecanol	36653-82-4	6.83	4.1×10^{-2}
Oleic acid eicosyl ester	22393-88-0	13.609	–
Octadecanoic acid	57-11-4	8.23	0.568–0.597
Octadecanoic acid 4-hydroxy-methyl ester	2420-38-4	–	–
Tridecanoic acid 4,8,12-trimethyl-methyl ester	5129-58-8	–	–
Succinic acid	110-15-6	-0.59	8.32×10^{4}
Triclosan	3380-34-5	4.76	12

Table 4. Log K_{OW} and water solubility of main additives of microplastics. Based on References [53–62].

In recent model analyses, however, it was shown that the effects of plastic on the bioaccumulation of POPs may be small, due to a lack of gradient between POPs in plastic and biota lipids, and that a cleaning mechanism is likely to dominate at higher log K_{OW} (octanol/water partition coefficient) values [44, 48, 49] (**Table 4**). In the case of additives, monomers or oligomers, which are components of the plastics, this issue has hardly been addressed. Many substances such as plasticizers may have biological effects even at low concentrations in the ng/L or μg/L range [50]. Although it has been argued that exposure to additives will probably be low because of the low diffusivities of the chemicals, bioaccumulation could increase the concentration in animal tissues. Moreover, POPs, like the bisphenols or nonylphenols found in plastics, have

been suggested to be a relevant environmental problem [51]. It has been reported that the concentrations of bisphenol A in wild freshwater fishes oscillated from undetected to 25.2 μg/kg biomass, while nonylphenol levels varied from 1.01 to 277 μg/kg [52]. So, the substances can enter and be accumulated by animals, and the log K_{OW} could give an idea of the behaviour of additives in aquatic environments and their solubility in water (**Table 4**).

5.1. Hydrocarbons

Hydrocarbons are organic compounds comprising only carbon and hydrogen atoms. The molecular structure comprises a frame of carbon and hydrogen atoms and grouped into saturated (straight, substituted and cyclic alkanes), unsaturated (alkenes with straight, branched and cyclic), halogenated and aromatic hydrocarbons. The hydrocarbons can be classified into two types—aliphatic and aromatic. Aliphatic hydrocarbons in turn can be classified into alkanes, alkenes and alkynes as link types that bind the carbon atoms. The general formulas of alkanes, alkenes and alkynes are C_nH_{2n+2}, C_nH_{2n} and C_nH_{2n-2}, respectively. Many alkanes with a chain length varying from C-11 to C-31 have been found in plastics from coastal debris [16]. These are other oligomers originating from polyolefins (polypropylene, polyethylene and poly(acetylene: styrene)) during recycling [63]. Octadecane ($n = 20/43$), hexadecane ($n = 19$), eicosane ($n = 18$), tetradecane ($n = 18$), heptacosane ($n = 14$), heptadecane ($n = 13$), pentadecane ($n = 11$), tetracosane ($n = 10$), docosane ($n = 8$), dodecane ($n = 7$), hexacosane ($n = 7$), 2,6,10-trimethyl-tetradecane ($n = 10$) and heptadecane, 3-methyl- ($n = 6$) were the most frequently detected in the plastic debris from near the coasts [16].

Linear alkanes, together with iso-alkanes, originate from the paraffin wax that is used as an external lubricant in PVC and other polymers, where they help the polymers to slide over other surfaces. Alkanes are also used as a solvents, such as hexane and heptane. Alkenes (squalene and others) and cycloalkenes are used as starting compounds for several additives and polymers and are formed as by-products during olefin polymerization.

Aromatic hydrocarbons, such as benzene and anthracene derivatives, have also been found in MP debris. Benzene is an important organic chemical compound used mainly as an intermediate to make other chemicals, mainly ethylbenzene, cumene, cyclohexane, nitrobenzene, and alkylbenzene. More than half of the entire benzene production is processed into ethylbenzene, a precursor of styrene, which is used to make polymers and plastics like polystyrene and expanded polystyrene. Around 20% of benzene production is used to manufacture cumene, which is needed to produce phenol and acetone for resins and adhesives. The plastics may also carry halogenated hydrocarbons, which have been considered as POPs and are of proven toxicity [64, 65].

5.2. UV stabilizers/absorbers

Benzophenone and its derivatives are used as photo-initiators in the UV curing of inks and as UV absorbers. These compounds absorb the harmful UV light that would eventually change the physical and optical properties of the polymer and make the material lose colour or fade. This substance can also be added to plastic packaging as a UV blocker to prevent photo-

degradation of the packaging polymers or contents. Its use allows manufacturers to package the product in clear glass or plastic [66] since, without the UV blocker, opaque or dark packaging would be required. These plastic additives are used in PP, PE (2–3%) and acrylonitrile, butadiene and styrene (ABS) copolymer products. Benzotriazole UV stabilizers (BUVS) are emerging contaminants that are mutagenic, toxic, pseudopersistent, bioaccumulated and show significant estrogenic activity [67–70]. Great amounts of BUVS have been detected in rivers from Japan and China coming from wastewater treatment plants [71–73]. Due to their common use, BUVS have been found in aquatics environments [69, 72, 74], organisms [71, 72, 74, 75], tap water and well water [76]. Recent findings in German rivers and previously reports suggest that BUVSs have a potential of long-range transport, similar to several POPs [74].

5.3. Antioxidants

Antioxidants are widely used in plastic polymers to delay oxidation and to improve polymer properties [77]. Several types of antioxidants can be used to prevent the aging of plastic, such as phenolic antioxidants, organophosphorus compounds and different amines. However, antioxidants can migrate from the plastics into the food and contaminate it during production or storage, potentially giving rise to food safety issues [78, 79]. Antioxidants are used in almost all commercial polymers in small amounts up to 2% (w/w) (20,000 mg/kg or ppm) [16]. The polymers can be oxidized during synthesis, processing, transfer or final use, resulting in loss of chemical, optical and mechanical properties, among others. Thermal oxidation results in the formation of free radicals that react with oxygen to form hydroperoxides. In order to inhibit the onset of thermal oxidation of polymers and/or slow down degradative processes, the antioxidant additives are added during manufacture, processing and/or during the manufacture of the products. In the specific case of the polypropylene, antioxidant additives are important because the chemical structure of this type of polyolefin tends to degrade easily. The plastic antioxidants identified in the literature are usually limited to the commonly used Irganox series (including Irganox 1010, Irganox 1076, Irganox 168) [80–84].

5.4. Plasticizers

Plastic as a material may contain a variety of chemicals, some potentially hazardous. Plasticizers, which are used to make the plastic soft and flexible, are mainly used in PVC, but they are detected in other polymer plastics. Several types of plasticizers are found in plastic debris, but phthalates predominate [85]. The phthalates found in plastics include dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DNOP) [86]. Their concentrations in different plastics vary widely; for example, in foodstuffs, the content of phthalates varies from 658 to 1610 ng/g fresh weight [87]. Phthalates are produced in large quantities around the world and are also widely used in cosmetics, plastics, carpets, building materials, toys, medical and cleaning products.

Several cross-sectional and case-control studies have reported an association between exposure to phthalates and the development of certain human allergies and respiratory diseases [88]. A recent systematic review based on less than ten relatively small ($N < 400$) studies found

that the findings from these studies are inconsistent, with both decreases in birthweight and null associations, and both longer and shorter gestational periods being recorded [89]. A prospective birth cohort study researched the association between butyl benzyl phthalate and an early-onset eczema, although not the late-onset eczema, finding that prenatal exposure to butylbenzyl phthalate may influence the risk of developing eczema in early childhood [90]. Three studies reported a positive relation between prenatal exposition and the risk of wheeze, asthma and respiratory infections in children aged 5–11 years [91–93], although, even here, there were inconsistencies concerning the phthalate congeners implicated [94].

On the other hand, phthalates have been related with hormone disorders [95], abortion [96], metabolic diseases [97], hormone disturbances, reprotoxicity or even suspected cancer [98–100]. Other plasticizers are often used as substitutes for phthalates, but their effects on the health are not always clear, usually because of the limited data available. Therefore, because the amount of plasticizers could increase the 50% of the total weight, and the possibility that these substances will leach when the plastics come into contact with seawater is greater [16], the substances called plasticizers should be considered in a hazard category and need be reviewed.

5.5. Lubricants

Usually, lubricants are used to minimize adhesion and viscosity of plastic polymers. Internal lubricants can facilitate the production process by providing lubrication at molecular level between the polymer chains [101]. Commonly, they are composed of an oil base accompanied by a variety of additives that confer desirable properties. Lubricants are based in one type of base oil, but in commercial requirements, it usually makes that a mixture are used [102]. n-Hexadecanoic acid, oleic acid, glycerol tricaprylate, isopropyl myristate, 1-eicosanol, 2-hexyl-1-decanol, octadecanamide, 4-methyl-benzenesulfonamide, 1-hexacosanol and decanedioic acid, bis(2-ethylhexyl) ester can be found in plastic debris [16]. The transfer of additives such as lubricants to the medium or to the substances which are in contact with the plastics has been reported previously [103].

5.6. Fuel

Chemicals like pentadactyl ester trichloroacetic acid, 1,10-[2-methyl-2-(phenylthio) cyclopropenylidene] bisbenzene and 2,4-dimethyl-4-octanol are often found in plastic debris [16]. These substances and others, like hexadecyl ester trichloroacetic acid, have been considered as fuel precursor based on plastic wastes additives, due to the large amount and variety of additives that plastics can contain [63]. Waste plastics are considered a promising source for fuel production because of their high combustion heat and their increasing availability in local communities [104].

5.7. Intermediates

In manufacture of plastics, it is normal to use stabilizers (DEHA or DEHP) and plasticizers that contain intermediate substances like hexanoic acid 2-ethyl-hexadecyl ester (HEHA), 2,3-

dihydroxypropyl ester hexadecanoic acid, hexadecanoic acid ethyl ester, behenic alcohol, nonanoic acid, pimaric acid, 3,5-di-tert-butyl-4-hydroxyphenyl propionic acid, abietic acid and dehydroabietic acid [16]. HEHA has been classified as belonging to reprotoxic category 3 by Council Directive 67/548/EEC [105].

5.8. Flame retardants

Flame retardants are a group of chemical compounds that are used in plastics with the aim of diminishing the flammability of combustible materials, like synthetic polymers and plastics. To make sure that flame retardants remain in the polymers, these compounds are designed to be stable for many years, which means they will remain in the environment long past the time when the material itself was used [106]. Thus, these compounds can enter aquatic environments via the atmospheric deposition of fine particles, direct discharges of municipal and industrial wastewater effluents, and through run-off and other human activities [107]. Flame retardants include α, β, γ-diastereoisomers of hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBP-A), anti- and syn-isomers of dechlorane plus (DP) and two novel compounds, decabromodiphenylethane (DBDPE) and 2-bis(2,4,6-tribromophenoxy) ethane (BTBPE). Among the most widely used flame retards are polybrominated diphenyl ethers, which have been in use since the late 1970s. Polybrominated diphenyl ethers are a class of brominated compounds widely used as flame retardants including in polymers such as low density polyethylene or silicone rubber [45, 108]. Polybrominated diphenyl ethers are very hydrophobic, with log K_{OW} above 5.5 and molecular weights (MW) in the range of 300–1000 g/mol which means that these compounds are likely to have diffusion coefficients significantly lower than those measured for polycyclic aromatic hydrocarbons and polychlorinated biphenyls. This implies that the polymer diffusion coefficients for these plastic additives used as flame retardants need to be taken into account when considering the risk posed by microplastic particle ingestion by marine organisms [109]. Many studies on polybrominated diphenyl ethers [110–118] have shown that these compounds are ubiquitous, toxic, persistent and bioaccumulated in the environment. As a result, some flame retardants have been prohibited in the USA and European Union [119, 120], such a penta- and octabrominated diphenyl ether. Nevertheless, new compounds have replaced the forbidden polybrominated diphenyl ethers, such as 1,2-bis(pentabromodiphenyl) ethane, which is used in solid plastics, wire, cable and electronics, high impact polystyrene and thermoplastics [121].

5.9. Monomers and oligomers

Bisphenol A (2,2-(4,4-dihydroxydiphenyl) propane) is used as a monomer in polycarbonate, for the production of polycarbonate plastics and epoxy resins. It has been found in samples of PE, PP and acrylate-styrene, where it is probably used as chain terminator, to finish the polymerization of polymers or as antioxidant for polymers or plasticizers [16]. Bisphenol A is also used to manufacture a great variety of products, including CDs, food can linings, thermal paper, safety helmets, plastic windows, car parts, adhesives, protective coatings, powder paints, and the sheathing of electrical and electronic parts [122]. As a result of its wide usage, bisphenol A is frequently detected in wastewaters [123].

Bisphenol A has been identified as an endocrine disruptor [124], and several studies have demonstrated reproductive, metabolic and neurodevelopmental problems in animals exposed to environmentally relevant levels of this substance [125–127]. In addition, an increased risk for cardiovascular disease, altered immune system activity, miscarriages, decreased birth-weight at term, metabolic problems and diabetes in adults, breast and prostate cancer, reproductive and sexual dysfunctions and cognitive and behavioural development in young children have been associated with the human exposure to bisphenol A [128–134].

It is known that plasticizers may have biological effects even at low concentrations in the ng/L range, especially for molluscs, crustaceans and amphibians [50]. Although it has been argued that one should expect levels of exposure to plastic additives to be low due to the low diffusivities of chemicals like bisphenol A or nonylphenol in plastics [51], as we said above, their bioaccumulation could play an important role, increasing physiological concentrations in the food chain. In an attempt to solve these problems, physicochemical processes for the removal of bisphenol A from wastewaters have been studied [135, 136]. However, possible solutions presented several problems related to the cost of chemicals, the generation of bisphenol A-containing sludge and the conditions necessary to optimize the bisphenol A elimination process. The most frequently detected metabolic products of the aerobic biodegradation pathway of bisphenol A include 4-hydroxyacetophenone and 4-hydroxybenzoic acid [137]. Both bisphenol A and 4-hydroxybenzoic acids have shown a certain degree of biodegradability [138], and these compounds are not expected to be persistent in an activated sludge system, although the information concerning 4-hydroxyacetophenone is scarce.

5.10. Others

Degradation products, antifogging, antiblocking, colouring, heat stabilizers, fatty acids and their derivatives have also been found in plastics debris [16]. This heterogeneous group includes 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, glycerol 1-palmitate, (Z)-13-docosenamide, 2,3-dichloro-1,10-biphenyl, trans-13-docosenamide, di-tert-dodecyl disulfide, 1-hexadecanol, 2,4-bis [2-(4-methoxyphenyl-2-propyl)] methoxybenzene, oleic acid eicosyl ester, octadecanoic acid, octadecanoic acid 4-hydroxy-methyl ester, octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester, tridecanoic acid 4,8,12-trimethyl-methyl ester, heptanedioic acid 4-(ethoxycarbonylmethylene)-diethyl ester and succinic acid [16]. Fatty acids and their esters could originate from several kinds of oils, such as coconut oil (lauric acid) or palm oil (palmitic acid), acids which, along with their esters, are usually used as internal lubricants. Besides, metallic salts of fatty acids are normally used as stabilizers and plasticizers in the production of the plastics.

Other substances can stick or bind to plastics, such as disinfectants, aromatic compounds, soaps used to clean the plastics. In this respect, triclosan (5-chloro-2-[2,4-dichloro-phenoxy]-phenol) is an additive that has been reported to be toxic [139–141]. Triclosan is an antimicrobial that is effective against bacteria of the adult oral cavity and skin. It is currently used in antibacterial soaps, deodorants, skin creams, toothpastes and plastics. Triclosan is an ionizable chlorinated biphenyl ether of low water solubility, with a pK_a of 8.1, and a vapour pressure of 4×10^{-6} mm Hg [139]. Triclosan readily bioaccumulates within aquatic organisms and has been

found to be toxic to fish. In larval fishes, exposure to triclosan disrupts a variety of developmental processes, impairs hatching success, and causes pericardial oedema, having the potential to cause subtle cardiac toxicity [142]. Browne et al. [47] showed that triclosan added to MPs diminished the ability of worms to engineer sediments and caused mortality, each by >55%, while PVC alone made worms >30% more susceptible to oxidative stress. Triclosan persists in water and is difficult to eliminate from wastewaters [143, 144]. The ingestion of MPs by organisms can transfer pollutants and additives (such as triclosan) to their tissues at concentrations sufficient to disrupt ecophysiological functions linked to health and biodiversity. Biomarkers of endocrine disruption found in fish indicated long-term exposure to estrogenic chemicals in the wastewater [145].

6. Effects on marine fish

The accumulation of microplastic waste could affect the functioning of marine ecosystems. However, the mechanisms by which these effects will be manifested have not been identified. Impacts on biota and marine environmental quality are well documented [146], with damage for the global economy estimated to be in the range of $13 billion per year [147].

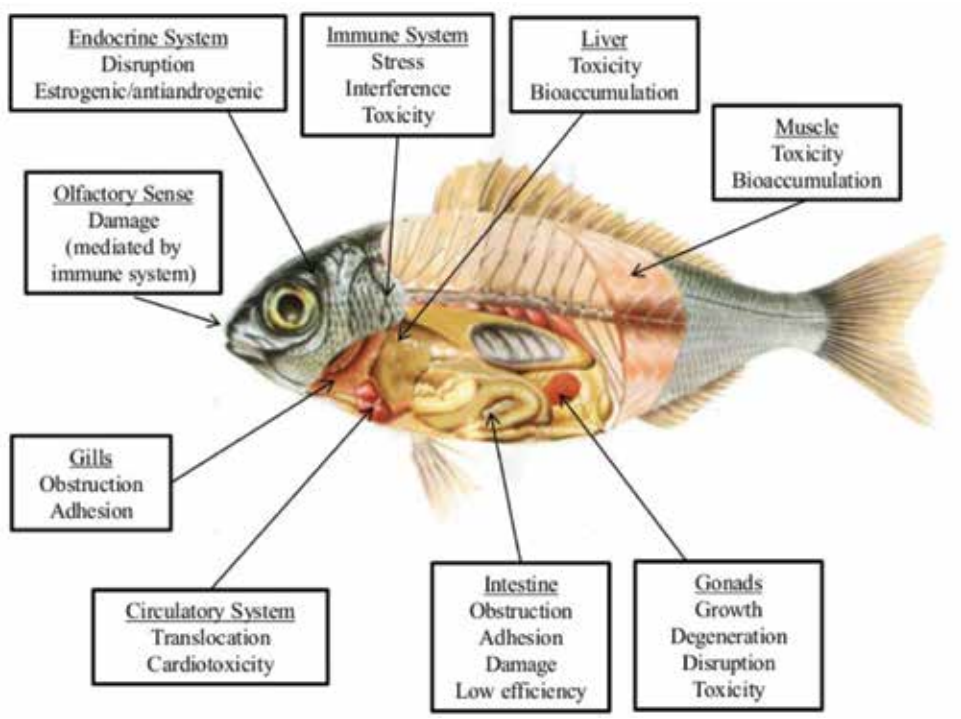

Figure 1. Principal effects of microplastics on fish.

Negative effects include entanglement in plastic wires or nets, or to ingestion, which has been reported in benthic invertebrates, birds, fish, mammals and turtles [148–151]. This is especially true for eggs, embryos and larvae of aquatic organisms, which are particularly vulnerable to water-borne pollutants owing to their limited ability to regulate their internal environment [152]. In particular, the early life stages of fishes are subjected to strong selection forces, driven by high rates of predator-induced mortality [153, 154]. So, it has been reported that there is a clear overlap between areas with high levels of microplastics pollution and the feeding grounds of fin whales in the Mediterranean Sea, which could mean that fin whales are subjected to a high level of exposure to MPs ingestion during feeding in the areas [155]. The bioaccumulation of MPs and the substances which they could carry seem to be an increasing problem due to MPs which has been detected from little fish species to the top of food web.

The ingestion of the MPs can influence marine animals in different ways (**Figure 1**). It can affect to the immune system, both chemically (caused by the substances that MPs might contain, absorb or release, which may be toxic) [156] and physically blocking the digestive organs and preventing the animals from feeding [157]. Ecology and behaviour could also be affected.

6.1. Immune system

Interactions between plastic microparticles and aquatic organisms have been reported, and several recent studies have addressed the effects of nanoplastic material on different organisms and their health status. This research suggests that nanoplastics can enter different organisms and may interact with the immune system [158–161].

In fish, cellular innate immunity effectors act as one of the first organ defences against various agents, which makes these effectors the possible target for interaction with nanoplastic particles. Neutrophil activation is critical for the host defences, and their function is a valuable tool to assess the health status of individuals and animal populations [162]. So, fish neutrophils can extravasate, migrate chemotactically, degranulate, release neutrophil extracellular traps and phagocytize particulate matter such as bacteria [163]. Hypotheses existed about the interactions between MPs or nanoplastics and the neutrophils until recently, it has been reported that polystyrene and polycarbonate nanoplastic can act as stressors to the innate immune response of fish [164]. Therefore, nanoplastic could potentially interfere with innate immune responses in fish populations by altering organismal defence mechanisms.

In addition, plastic fragments found in the marine habitat have been shown to absorb POPs, so effects on the immune system may be caused by particle toxicity, plastic-associated chemicals and absorbed environmental chemicals.

6.2. Disrupting effects

Evidence points to the potential role of microplastics as vectors of chemical pollutants, either used as additives during polymer synthesis, or adsorbed directly from seawater [27, 45, 165]. The hydrophobicity of organic xenobiotics and the surfaces of polymers facilitate the adsorption of the chemicals on MPs at concentrations with orders of magnitude higher than those usually detected in seawater [166].

Several of these plastic-associated chemicals have been linked to endocrine-disrupting effects in fish. Styrene [167], a monomer of several plastic types including polystyrene, rubber and acrylonitrile–butadiene–styrene, and bisphenol-A [168] a monomer of polycarbonate, can disrupt the endocrine system function, as mentioned above. In addition, there is evidence that UV stabilizers, phthalates and nonylphenol, additives to plastic, are estrogenic and/or antiandrogenic [169, 170]. Furthermore, chemicals historically known to promote adverse effects in the endocrine system functions, including heavy metals, organochlorine pesticides and petroleum hydrocarbons [171, 172], have been found attached to plastic debris around the world [173, 174].

The ingestion of plastic debris has been documented in fish [175, 176], which may introduce a 'cocktail' of endocrine-disrupting chemicals [47, 150, 177]. Significantly higher concentrations of several polybrominated diphenyl ethers, such as polychlorinated biphenyl congener (PCB#28) and the polycyclic aromatic hydrocarbon chrysene, have been recorded in Japanese medaka (*Oryzias latipes*) exposed to polyethylene that had been deployed in the marine environment compared to fish exposed to a virgin polyethylene and a control treatment [177].

Fish are useful as sensitive indicators of endocrine-disrupting chemicals in aquatic habitats, as exposure can result in changes in gonadal growth, gonadal degeneration, sex-specific gene protein and intersex induction [178]. Finally, recent research showed that ingestion of plastic debris at environmentally relevant concentrations may alter the endocrine system function in adults [179], where the presence of abnormal germ cell proliferation observed may be related to plastic. In this respect, ovary structure protein 1 (OSP1) gene has been proposed as a suitable indicator of the early stages of intersex development and suggested to be a more sensitive early-warning signal than histopathological observation [180].

6.3. Physiological

It has been shown in various marine organisms that ingestion of MPs occurs in animals with different feeding strategies and may negatively influence both the feeding activity and nutritional value, especially in species which cannot vary their food source [181, 182]. Different studies have pointed to the obstruction and damage of digestive tracts or even animals starving to death caused by stomachs filled with plastic [18]. In addition, MP ingestion by marine biota has been detected in benthic fish species [183, 184], and different sized plastic items were identified in the stomachs of three large pelagic fish in the Mediterranean Sea [185].

In a study made in Spanish coastal waters and which constitutes the first report of MPs ingestion by demersal fishes, red mullets (*Mullus barbatus*) from Barcelona presented the highest abundance of microplastics, followed by dogfish (*Scyliorhinus canicula*) from the Cantabrian coast and the Gulf of Cadiz, whereas dogfish from the Galician coast presented the lowest levels [186]. In agreement with previous studies, the detected MPs were mostly fibres (71%) [174, 184, 187], and the most frequent colour was black (51%) (**Table 5**).

Because of their small size, MPs may be ingested by marine organisms, regardless of their feeding mechanisms, and may enter their circulatory system and accumulate in different types of tissues, as has been proven in laboratory experiments [182]. These reported data, along with

the fact that MPs serve as dispersal vectors for invasive species [188] and the toxic and bioaccumulative substances bound to the plastics [149], together with the research that indicates that MPs may have the ability to enter and disseminate though the marine food web [189, 190], suggest grave ecological implications of microplastics across the food web.

Form	Percentage (%)
Fibre	71.0
Sphere	24.2
Film	3.2
Fragment	1.6

Table 5. Types of plastics found in fish and their relative abundance in Spanish coastal waters [188].

6.4. Behaviour

Behaviour is a crucial determinant for essential parameters such as overall health, growth, reproduction and survival [191]. During the life cycle of fish, a critical point is the early stage of development. Survival depends, in many cases, on the capacity of the organism to evade predators. An innate ability to detect and act accordingly is therefore vital [153, 154, 192].

In this regard, it has been suggest that olfactory sense in fish larvae could suffer damage mediated by an immunological response produced by the pollutant from microplastics. Lönnstedt and Eklöv [193] found that not only was crucial behaviour, such as activity and feeding, affected by microplastics, but that innate responses to olfactory threat cues were also impaired. Such a loss of predator avoidance behaviour greatly increased predator-induced mortality rates of larvae. Finally, survival of fishes could be seriously affected by the presence of MPs, with their significant impact on the life cycle of the fish.

7. Conclusion

Microplastics in the aquatic environment have been demonstrated to be a significant problem. The great amount of research on this topic, as well as the quantity of the results that describe the problem of MPs and their effects on fishes and aquatic life, have thrown some light on this issue. Among the effects that MPs have are stress, intestinal obstruction and the alteration of health, while further studies are in progress to ascertain the full potential risks of MPs in aquatic organisms with special attention paid to fish. A huge number of substances are added to plastics, which can bioaccumulate throughout the trophic chain. Besides the problems that MPs represent for marine life in general, the MPs could begin act as disruptors of the welfare and health of fishes, both wild and cultivated. This is clearly a growing problem not only for the environment but also for human health. For these reasons, further efforts are needed to know the exact effects that microplastics, and their constitutive and adsorbed contaminants, may have on aquatic environments.

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References

[1] Plastics: The Facts 2015: An analysis of European latest plastics production, demand and waste data. [Internet]. 2015. Available from: http://www.corepla.it/documenti/5f2fa32a-7081-416f-8bac-2efff3ff2fbd/Plastics+TheFacts+2015.pdf [Accessed: 2016-06-29]

[2] Eubeler JP, Bernhard M, Knepper TP. Environmental biodegradation of synthetic polymers II. Biodegradation of different polymer groups. TrAC Trends Anal Chem. 2010;29:84–100

[3] Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc Lond B Biol Sci. 2009;364:1985–1998. doi:10.1098/rstb.2008.0205

[4] Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, et al. Lost at sea: where is all the plastic? Science. 2004;304:838. doi:10.1126/science.1094559

[5] Andrady AL. Microplastics in the marine environment. Mar Pollut Bull. 2011;62:1596–1605. doi:10.1016/j.marpolbul.2011.05.030

[6] Gregory MR, Ryan PG. Pelagic plastics and other seaborne persistent synthetic debris: a review of southern hemisphere perspectives. In: Coe JM, Rogers DB, editors. New York: Springer Series on Environmental Management; 1997. p. 49–66

[7] Zarfl C, Matthies M. Are marine plastic particles transport vectors for organic pollutants to the Arctic? Mar Pollut Bull. 2010;60:1810–1814. doi:10.1016/j.marpolbul.2010.05.026

[8] Roy PK, Hakkarainen M, Varma IK, Albertsson A-C. Degradable polyethylene: fantasy or reality. Environ Sci Technol. 2011;45:4217–4227. doi:10.1021/es104042f

[9] Gregory MR, Andrady AL, editors. Plastics in the marine environment, in plastics and the environment. Wiley, A. L. Andrad, Inc., Hoboken, NJ, USA. 2003. doi: 10.1002/0471721557.ch10

[10] Frankel EN. In search of better methods to evaluate natural antioxidants and oxidative stability of food lipids. Trends Food Sci Technol. 1993;4:220–225

[11] Betts K. Why small plastic particles may pose a big problem in the oceans. Environ Sci Technol. 2008;42:8995

[12] Moore CJ. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ Res. 2008;108:131–139

[13] Koelmans AA, Besseling E, Shim W. Nanoplastics in the aquatic environment. Critical review. In: Bergmann M, Gutow L, Klages M, editors. Marine Anthropog Litter. 2015;3:325–340. doi: 10.1007/978-3-319-16510-3_12

[14] Cozar A, Echevarria F, Gonzalez-Gordillo JI, Irigoien X, Ubeda B, Hernandez-Leon S, et al. Plastic debris in the open ocean. Proc Natl Acad Sci. 2014;111:10239–10244. doi: 10.1073/pnas.1314705111

[15] Andrady AL, Neal MA, Andersen ME, Clewell HJ, Tan Y-M, Butenhoff JL, et al. Applications and societal benefits of plastics. Philos Trans R Soc Lond B Biol Sci. 2009;364:1977–1984. doi:10.1098/rstb.2008.0304

[16] Rani M, Shim WJ, Han GM, Jang M, Al-Odaini NA, Song YK, et al. Qualitative analysis of additives in plastic marine debris and its new products. Arch Environ Contam Toxicol. 2015;69:352–66. doi:10.1007/s00244-015-0224-x

[17] Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ Sci Technol. 2012;46:3060–3075. doi:10.1021/es2031505

[18] Kühn S, Bravo Rebolledo EL, van Franeker JA. Deleterious effects of litter on marine life. Mar. Anthropog. Litter, Cham: Springer International Publishing; 2015. 75–116 p. doi:10.1007/978-3-319-16510-3_4

[19] Browne MA, Crump P, Niven SJ, Teuten EL, Tonkin A, Galloway T, et al. Accumulations of microplastic on shorelines worldwide: sources and sinks. Environ Sci Technol. 2011;45(21):9175–9179. doi:10.1021/es201811s

[20] Li WC, Tse HF, Fok L. Plastic waste in the marine environment: a review of sources, occurrence and effects. Sci Total Environ. 2016;566:333–349. doi:10.1016/j.scitotenv.2016.05.084

[21] Yonkos LT, Friedel EA, Perez-Reyes AC, Ghosal S, Arthur CD. Microplastics in four estuarine rivers in the Chesapeake Bay, U.S.A. Environ Sci Technol. 2014;48:14195–14202. doi:10.1021/es5036317

[22] Castañeda R, Avlijas S. Microplastic pollution in St. Lawrence River sediments. Can J Fish Aquat Sci. 2014;5:1–5

[23] Mani T, Hauk A, Walter U, Burkhardt-Holm P. Microplastics profile along the Rhine River. Sci Rep. 2015;5:17988. doi:10.1038/srep17988

[24] Lechner A, Keckeis H, Lumesberger-Loisl F, Zens B, Krusch R, Tritthart M, et al. The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ Pollut. 2014;188:177–181. doi:10.1016/j.envpol.2014.02.006

[25] Sundt P, Schulze P-E, Syversen F. Sources of microplastics-pollution to the marine environment, Report M-321-2015. 2014

[26] Magnusson K, Noren F. Screening of microplastic particles in and down-stream a wastewater treatment plant. 2014;55:1–22

[27] Engler RE. The complex interaction between marine debris and toxic chemicals in the ocean. Environ Sci Technol. 2012;46:12302–12315. doi:10.1021/es3027105

[28] Thompson RC, Moore CJ, vom Saal FS, Swan SH. Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc Lond B Biol Sci. 2009;364:2153–2166. doi:10.1098/rstb.2009.0053

[29] Driedger AGJ, Dürr HH, Mitchell K, Van Cappellen P. Plastic debris in the Laurentian Great Lakes: a review. J Great Lakes Res. 2015;41:9–19. doi:10.1016/j.jglr.2014.12.020

[30] Duis K, Coors A. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ Sci Eur. 2016;16(1): 273. doi:10.1186/s12302-015-0069-y

[31] Lagarde F, Olivier O, Zanella M, Daniel P, Hiard S, Caruso A. Microplastic interactions with freshwater microalgae: hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ Pollut. 2016;215:331–339. doi:10.1016/j.envpol.2016.05.006

[32] Long M, Moriceau B, Gallinari M, Lambert C, Huvet A, Raffray J, et al. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar Chem. 2015;175:39–46. doi:10.1016/j.marchem.2015.04.003

[33] Eriksen M, Maximenko N, Thiel M, Cummins A, Lattin G, Wilson S, et al. Plastic pollution in the South Pacific subtropical gyre. Mar Pollut Bull. 2013;68:71–76. doi:10.1016/j.marpolbul.2012.12.021

[34] Goldstein MC, Titmus AJ, Ford M, Derraik J, Barnes D, Galgani F, et al. Scales of spatial heterogeneity of plastic marine debris in the Northeast Pacific Ocean. PLoS One. 2013;8(11):e80020. doi:10.1371/journal.pone.0080020

[35] Shaw DG, Day RH. Colour- and form-dependent loss of plastic micro-debris from the North Pacific Ocean. Mar Pollut Bull. 1994;28:39–43. doi:10.1016/0025-326X(94)90184-8

[36] Doyle MJ, Watson W, Bowlin NM, Sheavly SB. Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean. Mar Environ Res. 2011;71:41–52. doi:10.1016/j.marenvres.2010.10.001

[37] Desforges J-PW, Galbraith M, Dangerfield N, Ross PS. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. Mar Pollut Bull. 2014;79:94–99. doi:10.1016/j.marpolbul.2013.12.035

[38] Lusher AL, Burke A, O'Connor I, Officer R. Microplastic pollution in the Northeast Atlantic Ocean: validated and opportunistic sampling. Mar Pollut Bull. 2014;88:325–333. doi:10.1016/j.marpolbul.2014.08.023

[39] Reddy MS, Shaik Basha, Adimurthy S, Ramachandraiah G. Description of the small plastics fragments in marine sediments along the Alang-Sosiya ship-breaking yard, India. Estuar Coast Shelf Sci. 2006;68:656–660. doi:10.1016/j.ecss.2006.03.018

[40] Collignon A, Hecq J-H, Glagani F, Voisin P, Collard F, Goffart A. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar Pollut Bull. 2012;64:861–864. doi:10.1016/j.marpolbul.2012.01.011

[41] Fossi MC, Panti C, Guerranti C, Coppola D, Giannetti M, Marsili L, et al. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*). Mar Pollut Bull. 2012;64:2374–2379. doi:10.1016/j.marpolbul.2012.08.013

[42] Mascia L. The role of additives in plastics. M PolymSci Pol Lett. 1975;13 (10):632. doi:10.1002/pol.1975.130131020

[43] Koelmans AA, Besseling E, Foekema EM. Leaching of plastic additives to marine organisms. Environ Pollut. 2014;187:49–54. doi:10.1016/j.envpol.2013.12.013

[44] Gouin T, Roche N, Lohmann R, Hodges G. A thermodynamic approach for assessing the environmental exposure of chemicals absorbed to microplastic. Environ Sci Technol. 2011;45:1466–1472. doi:10.1021/es1032025

[45] Teuten EL, Saquing JM, Knappe DRU, Barlaz MA, Jonsson S, Björn A, et al. Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc Lond B Biol Sci. 2009;364:2027–2045. doi:10.1098/rstb.2008.0284

[46] Hammer J, Kraak MHS, Parsons JR. Plastics in the marine environment: the dark side of a modern gift. Rev Environ Contam Toxicol. 2012;220:1–44. doi:10.1007/978-1-4614-3414-6_1

[47] Browne MA, Niven SJ, Galloway TS, Rowland SJ, Thompson RC. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr Biol. 2013;23:2388–2392. doi:10.1016/j.cub.2013.10.012

[48] Koelmans AA, Besseling E, Wegner A, Foekema EM. Plastic as a carrier of POPs to aquatic organisms: a model analysis. Environ Sci Technol. 2013;47:7812–7820. doi: 10.1021/es401169n

[49] Koelmans AA, Besseling E, Wegner A, Foekema EM. Correction to plastic as a carrier of POPs to aquatic organisms. A model analysis. Environ Sci Technol. 2013;47:8992–8993

[50] Oehlmann J, Schulte-Oehlmann U, Kloas W, Jagnytsch O, Lutz I, Kusk KO, et al. A critical analysis of the biological impacts of plasticizers on wildlife. Philos Trans R Soc Lond B Biol Sci. 2009;364:2047–2062. doi:10.1098/rstb.2008.0242

[51] Berens AR. Predicting the migration of endocrine disrupters from rigid plastics. Polym Eng Sci. 1997;37:391–395. doi:10.1002/pen.11681

[52] Lee C-C, Jiang L-Y, Kuo Y-L, Chen C-Y, Hsieh C-Y, Hung C-F, et al. Characteristics of nonylphenol and bisphenol A accumulation by fish and implications for ecological and human health. Sci Total Environ. 2015;502:417–425. doi:10.1016/j.scitotenv.2014.09.042

[53] Staples CA, Parkerton TF, Peterson DR. A risk assessment of selected phthalate esters in North American and Western European surface waters. Chemosphere. 2000;40:885–891. doi:10.1016/S0045-6535(99)00315-X

[54] Santa Cruz Biotechnology, Inc. [Internet]. 2007. Available from: http://www.scbt.com. [Accessed: 2016-07-01]

[55] European chemical agency [Internet]. 2007. Available from: http://echa.europa.eu/es. [Accessed: 2016-07-01]

[56] National Library of Medicine. PUBMED [Internet]. 2000. Available from: http://pubchem.ncbi.nlm.nih.gov. [Accessed: 2016-07-01]

[57] ChemSpider. Royal Society of Chemistry [Internet]. 2015. Available from: http://www.chemspider.com. [Accessed: 2016-07-01]

[58] Guerra P, Eljarrat E, Barceló D. Determination of halogenated flame retardants by liquid chromatography coupled to mass spectrometry. TrAC Trends Anal Chem. 2011;30:842–855. doi:10.1016/j.trac.2011.01.018

[59] Anderson DR, Lusty EB. Acute toxicity and bioaccumulation of chloroform to four species of freshwater fish. Batelle Pacific Northwest Laboratories, US Nuclear Regulatory Commission, Richland, Washington, Report No. CR-0893. 1980

[60] Chain EFSA Panel on Contaminants in the Food. Scientific opinion on polybrominated biphenyls (PBBs) in food. Eur Food Saf Auth. 2010;8:1–151. doi:10.2903/j.efsa.2010.1789

[61] Palm A, Cousins IT, Mackay D, Tysklind M, Metcalfe C, Alaee M. Assessing the environmental fate of chemicals of emerging concern: a case study of the polybrominated diphenyl ethers. Environ Pollut. 2002;117:195–213

[62] Toxicological profile for polybrominated biphenyls and polybrominated diphenyl ethers. ATSDR, Atlanta, GA. Agency Toxic Subst Dis Regist. [Internet]. 2004. Available from: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=529&tid=94. [Accessed: 2016-07-01]

[63] Rashid MM, Sarker M. Waste polyethylene terephthalate (PETE) and polystyrene (PS) into fuel. Int J Sci Technol Res. 2013;2:176–189

[64] Rogan WJ, Gladen BC. Neurotoxicology of PCBs and related compounds. Neurotoxicology. 1992;13:27–35

[65] Evangelista de Duffard AM, Duffard R. Behavioral toxicology, risk assessment, and chlorinated hydrocarbons. Environ Health Perspect. 1996;104:353–360

[66] Dornath PJ. Analysis of chemical leaching from common consumer plastic bottles under high stress conditions [thesis] Oregon State University. University Honors College, USA; 2010

[67] Kawamura Y, Ogawa Y, Nishimura T, Kikuchi Y, Nishikawa J, Nishihara T, et al. Estrogenic activities of UV stabilizers used in food contact plastics and benzophenone derivatives tested by the Yeast two-hybrid assay. J Heal Sci. 2003;49:205–212

[68] Morohoshi K, Yamamoto H, Kamata R, Shiraishi F, Koda T, Morita M. Estrogenic activity of 37 components of commercial sunscreen lotions evaluated by *in vitro* assays. Toxicol *In Vitro*. 2005;19:457–469. doi:10.1016/j.tiv.2005.01.004

[69] Fent K, Kunz PY, Gomez E. UV Filters in the aquatic environment induce hormonal effects and affect fertility and reproduction in fish. Chim Int J Chem. 2008;62:368–375. doi:10.2533/chimia.2008.368

[70] Kim J-W, Chang K-H, Isobe T, Tanabe S. Acute toxicity of benzotriazole ultraviolet stabilizers on freshwater crustacean (*Daphnia pulex*). J Toxicol Sci. 2011;36:247–251

[71] Nakata H, Shinohara R, Murata S, Watanabe M. Detection of benzotriazole UV stabilizers in the blubber of marine mammals by gas chromatography-high resolution mass spectrometry (GC-HRMS). J Environ Monit. 2010;12:2088–2092. doi:10.1039/c0em00170h

[72] Kameda Y, Kimura K, Miyazaki M. Occurrence and profiles of organic sun-blocking agents in surface waters and sediments in Japanese rivers and lakes. Environ Pollut. 2011;159:1570–1576. doi:10.1016/j.envpol.2011.02.055

[73] Zhang Z, Ren N, Li Y-F, Kunisue T, Gao D, Kannan K. Determination of benzotriazole and benzophenone UV filters in sediment and sewage sludge. Environ Sci Technol. 2011;45:3909–3916. doi:10.1021/es2004057

[74] Wick A, Jacobs B, Kunkel U, Heininger P, Ternes TA. Benzotriazole UV stabilizers in sediments, suspended particulate matter and fish of German rivers: new insights into

occurrence, time trends and persistency. Environ Pollut. 2016;212:401–412. doi:10.1016/j.envpol.2016.01.024

[75] Gago-Ferrero P, Díaz-Cruz MS, Barceló D. Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. J Chromatogr A. 2013;1286:93–101. doi:10.1016/j.chroma.2013.02.056

[76] Díaz-Cruz MS, Gago-Ferrero P, Llorca M, Barceló D. Analysis of UV filters in tap water and other clean waters in Spain. Anal Bioanal Chem. 2012;402:2325–2333. doi:10.1007/s00216-011-5560-8

[77] Pezo D, Salafranca J, Nerín C. Development of an automatic multiple dynamic hollow fibre liquid-phase microextraction procedure for specific migration analysis of new active food packagings containing essential oils. J Chromatogr A. 2007;1174:85–94. doi:10.1016/j.chroma.2007.08.033

[78] Lau OW, Wong SK. Contamination in food from packaging material. J Chromatogr A. 2000;882:255–270

[79] Heiserman WM, Can SZ, Walker RA, Begley TH, Limm W. Interfacial behavior of common food contact polymer additives. J Colloid Interface Sci. 2007;311:587–594. doi:10.1016/j.jcis.2007.03.047

[80] Bodai Z, Szabó BS, Novák M, Hámori S, Nyiri Z, Rikker T, et al. Analysis of potential migrants from plastic materials in milk by liquid chromatography-mass spectrometry with liquid-liquid extraction and low-temperature purification. J Agric Food Chem. 2014;62:10028–10037. doi:10.1021/jf503110v

[81] Farajzadeh MA, Bahram M, Jönsson JA. Dispersive liquid-liquid microextraction followed by high-performance liquid chromatography-diode array detection as an efficient and sensitive technique for determination of antioxidants. Anal Chim Acta. 2007;591:69–79. doi:10.1016/j.aca.2007.03.040

[82] Sobhi HR, Kashtiaray A, Farahani H, Farahani MR. Quantitation of antioxidants in water samples using ionic liquid dispersive liquid-liquid microextraction followed by high-performance liquid chromatography-ultraviolet detection. J Sep Sci. 2011;34:77–82. doi:10.1002/jssc.201000526

[83] Nerín C, Salafranca J, Cacho J, Rubio C. Separation of polymer and on-line determination of several antioxidants and UV stabilizers by coupling size-exclusion and normal-phase high-performance liquid chromatography columns. J Chromatogr A. 1995;690:230–236. doi:10.1016/0021-9673(94)01132-X

[84] Lin Q-B, Li B, Song H, Li X-M. Determination of 7 antioxidants, 8 ultraviolet absorbents, and 2 fire retardants in plastic food package by ultrasonic extraction and ultra performance liquid chromatography. J Liq Chromatogr Relat Technol. 2011;53:1026–1035. doi:10.1093/chromsci/bmu159

[85] Pivnenko K, Eriksen MK, Martín-Fernández JA, Eriksson E, Astrup TF. Recycling of plastic waste: presence of phthalates in plastics from households and industry. Waste Manag. 2016;54:44–52. doi:10.1016/j.wasman.2016.05.014

[86] He M, Yang C, Geng R, Zhao X, Hong L, Piao X, et al. Monitoring of phthalates in foodstuffs using gas purge microsyringe extraction coupled with GC-MS. Anal Chim Acta. 2015;879:63–68. doi:10.1016/j.aca.2015.02.066

[87] Phuong NN, Zalouk-Vergnoux A, Poirier L, Kamari A, Châtel A, Mouneyrac C, et al. Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ Pollut. 2016;211:111–123. doi:10.1016/j.envpol.2015.12.035

[88] Jurewicz J, Hanke W. Exposure to phthalates: reproductive outcome and children health. A review of epidemiological studies. Int J Occup Med Environ Health. 2011;24:115-141. doi:10.2478/s13382-011-0022-2

[89] Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25:247–254. doi:10.1097/MOP.0b013e32835e1eb6

[90] Just AC, Whyatt RM, Perzanowski MS, Calafat AM, Perera FP, Goldstein IF, et al. Prenatal exposure to butylbenzyl phthalate and early eczema in an urban cohort. Environ Health Perspect. 2012;120:1475–1480. doi:10.1289/ehp.1104544

[91] Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gómez A, Luque N, et al. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. J Allergy Clin Immunol. 2015;135:370–378. doi:10.1016/j.jaci.2014.09.030

[92] Whyatt RM, Perzanowski MS, Just AC, Rundle AG, Donohue KM, Calafat AM, et al. Asthma in inner-city children at 5–11 years of age and prenatal exposure to phthalates: the Columbia center for children's environmental health cohort. Environ Health Perspect. 2014;122:1141–1146. doi:10.1289/ehp.1307670

[93] Ku HY, Su PH, Wen HJ, Sun HL, Wang CJ, Chen HY, et al. Prenatal and postnatal exposure to phthalate esters and asthma: a 9-year follow-up study of a Taiwanese birth cohort. PLoS One. 2015;10:e0123309. doi:10.1371/journal.pone.0123309

[94] Vrijheid M, Casas M, Gascon M, Valvi D, Nieuwenhuijsen M. Environmental pollutants and child health—A review of recent concerns. Int J Hyg Environ Health. 2016;219:331–342. doi:10.1016/j.ijheh.2016.05.001

[95] Minatoya M, Naka Jima S, Sasaki S, Araki A, Miyashita C, Ikeno T, et al. Effects of prenatal phthalate exposure on thyroid hormone levels, mental and psychomotor development of infants: the Hokkaido study on environment and children's health. Sci Total Environ. 2016;565:1037–1043. doi:10.1016/j.scitotenv.2016.05.098

[96] Yi H, Gu H, Zhou T, Chen Y, Wang G, Jin Y, et al. A pilot study on association between phthalate exposure and missed miscarriage. Eur Rev Med Pharmacol Sci. 2016;20:1894–902

[97] Mráz M, Svačina Š, Kotrlíková E, Piecha R, Vrbík K, Pavloušková J, et al. Potential sources of phthalates and bisphenol A and their significance in the development of metabolic diseases. Casopís Lékaru Ceských. 2016;155:11–15

[98] Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect. 1995;103:582–587

[99] Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen Z, et al. Phthalate exposure and human semen parameters. Epidemiology. 2003;14:269–277

[100] Hauser R, Calafat AM. Phthalates and human health. Occup Environ Med. 2005;62:806–818. doi:10.1136/oem.2004.017590

[101] Yarimitsu S, Moro T, Kyomoto M, Watanabe K, Tanaka S, Ishihara K, et al. Influences of dehydration and rehydration on the lubrication properties of phospholipid polymer-grafted cross-linked polyethylene. Proc Inst Mech Eng H. 2015;229:506–514. doi: 10.1177/0954411915588969

[102] Pomelli C, Ghilardi T, Chiappe C, de Angelis A, Calemma V. Alkylation of methyl linoleate with propene in ionic liquids in the presence of metal salts. Molecules. 2015;20:21840–21853. doi:10.3390/molecules201219805

[103] Fordham PJ, Gramshaw JW, Crews HM, Castle L. Element residues in food contact plastics and their migration into food simulants, measured by inductively-coupled plasma-mass spectrometry. Food Addit Contam. 1995;12:651–669. doi: 10.1080/02652039509374354

[104] Kumar PS, Sankaranarayanan G. Investigation on environmental factors of waste plastics into oil and its emulsion to control the emission in DI diesel engine. Ecotoxicol Environ Saf. 2016. S0147-6513:30021-30025. doi:10.1016/j.ecoenv.2016.01.021

[105] Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substance. English special edition: Series I. 1967. p. 234–256

[106] Eljarrat E, Barceló D, editors. The handbook of environmental chemistry. ed Berlin Heidelberg: Springer-Verlag; 2011. 290 p. doi:10.1016/0143-1471(82)90111-8

[107] Al-Odaini NA, Yim UH, Kim NS, Shim WJ, Hong SH. Isotopic dilution determination of emerging flame retardants in marine sediments by HPLC-APCI-MS/MS. Anal Methods. 2013;5:1771. doi:10.1039/c3ay25963c

[108] Reynier A, Dole P, Humbel S, Feigenbaum A. Diffusion coefficients of additives in polymers. I. Correlation with geometric parameters. J Appl Polym Sci. 2001;82:2422–2433. doi:10.1002/APP.2093

[109] Narváez Valderrama JF, Baek K, Molina FJ, Allan IJ. Implications of observed PBDE diffusion coefficients in low density polyethylene and silicone rubber. Environ Sci Process Impacts. 2016;18:87–94. doi:10.1039/c5em00507h

[110] Zhu L, Hites RA. Brominated flame retardants in tree bark from North America. Environ Sci Technol. 2006;40:3711–3716

[111] Kim GB, Stapleton HM. PBDEs, methoxylated PBDEs and HBCDs in Japanese common squid (*Todarodes pacificus*) from Korean offshore waters. Mar Pollut Bull. 2010;60:935–940. doi:10.1016/j.marpolbul.2010.03.025

[112] Hu G, Xu Z, Dai J, Mai B, Cao H, Wang J, et al. Distribution of polybrominated diphenyl ethers and decabromodiphenylethane in surface sediments from Fuhe River and Baiyangdian Lake, North China. J Environ Sci (China). 2010;22:1833–1839

[113] Hong SH, Kannan N, Jin Y, Won JH, Han GM, Shim WJ. Temporal trend, spatial distribution, and terrestrial sources of PBDEs and PCBs in Masan Bay, Korea. Mar Pollut Bull. 2010;60:1836–1841. doi:10.1016/j.marpolbul.2010.05.023

[114] Boer J de, Allchin C, Law R, Zegers B, Boon JP. Method for the analysis of polybrominated diphenylethers in sediments and biota. Trends Anal Chem. 2001;20:591–599

[115] Sellstrom U, B. Jansson. Analysis of tetrabromobisphenol A in a product and environmental samples. Chemosphere. 1995;31:3085–3092

[116] Sjödin A, Carlsson H, Thuresson K, Sjölin S, Bergman A, Ostman C. Flame retardants in indoor air at an electronics recycling plant and at other work environments. Environ Sci Technol. 2001;35:448–454

[117] Oberg K, Warman K, Oberg T. Distribution and levels of brominated flame retardants in sewage sludge. Chemosphere. 2002;48:805–809

[118] Kierkegaard A, Björklund J, Fridén U. Identification of the flame retardant decabromodiphenyl ethane in the environment. Environ Sci Technol. 2004;38:3247–3253

[119] California State Assembly, Assembly Bill no. 302, California State. 2003

[120] Viganò L, Mascolo G, Roscioli C. Emerging and priority contaminants with endocrine active potentials in sediments and fish from the River Po (Italy). Environ Sci Pollut R. 2015;22:14050–14066. doi:10.1007/s11356-015-4388-8

[121] Covaci A, Harrad S, Abdallah MA-E, Ali N, Law RJ, Herzke D, et al. Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. Environ Int. 2011;37:532–556. doi:10.1016/j.envint.2010.11.007

[122] Staples CA, Dorn PB, Klecka GM, O'Block ST, Harris LR. A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere. 1998;36:2149–2173

[123] Melcer H, Klecka G. Treatment of wastewaters containing bisphenol A: state of the science review. Water Environ Res. 2011;83:650–666

[124] Omoike A, Wacker T, Navidonski M. Biodegradation of bisphenol A by Heliscus lugdunensis, a naturally occurring hyphomycete in freshwater environments. Chemosphere. 2013;91:1643–1647. doi:10.1016/j.chemosphere.2012.12.045

[125] Salian S, Doshi T, Vanage G. Perinatal exposure of rats to Bisphenol A affects the fertility of male offspring. Life Sci. 2009;85:742–52. doi:10.1016/j.lfs.2009.10.004

[126] Wei J, Lin Y, Li Y, Ying C, Chen J, Song L, et al. Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. Endocrinology. 2011;152:3049–3061. doi:10.1210/en.2011-0045

[127] Wolstenholme JT, Taylor JA, Shetty SRJ, Edwards M, Connelly JJ, Rissman EF. Gestational exposure to low dose bisphenol A alters social behavior in juvenile mice. PLoS One. 2011;6:e25448. doi:10.1371/journal.pone.0025448

[128] Braun JM, Kalkbrenner AE, Calafat AM, Yolton K, Ye X, Dietrich KN, et al. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics. 2011;128:873–882. doi:10.1542/peds.2011-1335

[129] Braun JM, Yolton K, Dietrich KN, Hornung R, Ye X, Calafat AM, et al. Prenatal bisphenol A exposure and early childhood behavior. Environ Health Perspect. 2009;117:1945–1952. doi:10.1289/ehp.0900979

[130] Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, et al. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA. 2008;300:1303–1310. doi:10.1001/jama.300.11.1303

[131] Clayton EMR, Todd M, Dowd JB, Aiello AE. The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006. Environ Health Perspect. 2011;119:390–396. doi:10.1289/ehp.1002883

[132] Li D, Zhou Z, Qing D, He Y, Wu T, Miao M, et al. Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. Hum Reprod. 2010;25:519–527. doi:10.1093/humrep/dep381

[133] Miao M, Yuan W, Zhu G, He X, Li D-K. In utero exposure to bisphenol-A and its effect on birth weight of offspring. Reprod Toxicol. 2011;32:64–68. doi:10.1016/j.reprotox.2011.03.002

[134] Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K. Exposure to bisphenol A is associated with recurrent miscarriage. Hum Reprod. 2005;20:2325–2329. doi:10.1093/humrep/deh888

[135] Zeng G, Zhang C, Huang G, Yu J, Wang Q, Li J, et al. Adsorption behavior of bisphenol A on sediments in Xiangjiang River, Central-south China. Chemosphere. 2006;65:1490–9. doi:10.1016/j.chemosphere.2006.04.013

[136] Xiaoying M, Guangming Z, Chang Z, Zisong W, Jian Y, Jianbing L, et al. Characteristics of BPA removal from water by PACl-Al13 in coagulation process. J Colloid Interface Sci. 2009;337:408–413. doi:10.1016/j.jcis.2009.05.052

[137] Gao J, Ellis LBM, Wackett LP. The university of Minnesota biocatalysis/biodegradation database: improving public access. Nucleic Acids Res. 2010;38:488–491. doi: 10.1093/nar/gkp771

[138] Stasinakis AS, Petalas A V, Mamais D, Thomaidis NS. Application of the OECD 301F respirometric test for the biodegradability assessment of various potential endocrine disrupting chemicals. Bioresour Technol. 2008;99:3458–3467. doi:10.1016/j.biortech. 2007.08.002

[139] Orvos DR, Versteeg DJ, Inauen J, Capdevielle M, Rothenstein A, Cunningham V. Aquatic toxicity of triclosan. Environ Toxicol Chem. 2002;21:1338–1349

[140] Ishibashi H, Matsumura N, Hirano M, Matsuoka M, Shiratsuchi H, Ishibashi Y, et al. Effects of triclosan on the early life stages and reproduction of medaka *Oryzias latipes* and induction of hepatic vitellogenin. Aquat Toxicol. 2004;67:167–179. doi:10.1016/ j.aquatox.2003.12.005

[141] Smith GR, Burgett AA. Effects of three organic wastewater contaminants on American toad, Bufo americanus, tadpoles. Ecotoxicology. 2005;14:477–482

[142] Saley A, Hess M, Miller K, Howard D, King-Heiden TC. Cardiac toxicity of Triclosan in developing zebrafish. Zebrafish.2016; ISSN (Online) 1557–8542. doi:10.1089/zeb. 2016.1257

[143] Davis EF, Klosterhaus SL, Stapleton HM. Measurement of flame retardants and triclosan in municipal sewage sludge and biosolids. Environ Int. 2012;40:1–7. doi: 10.1016/j.envint.2011.11.008

[144] McAvoy DC, Schatowitz B, Jacob M, Hauk A, Eckhoff WS. Measurement of triclosan in wastewater treatment systems. Environ Toxicol Chem. 2002;21:1323–1329

[145] Barber LB, Loyo-Rosales JE, Rice CP, Minarik TA, Oskouie AK. Endocrine disrupting alkylphenolic chemicals and other contaminants in wastewater treatment plant effluents, urban streams, and fish in the Great Lakes and Upper Mississippi River Regions. Sci Total Environ. 2015;517:195–206. doi:10.1016/j.scitotenv.2015.02.035

[146] Ivar Do Sul JA, Costa MF. The present and future of microplastic pollution in the marine environment. Environ Pollut. 2014;185:352–364. doi:10.1016/j.envpol.2013.10.036

[147] UNEP, Plastic waste causes financial damage of US$13 billion to marine ecosystems each year as concern grows over microplastics. [Internet] Available from: http:// www.unep.org/newscentre/ [accessed March 29, 2016]

[148] Laist DW. Marine Debris. In: Coe, JM. Rogers, DB, editors. New York, NY: Springer; 1997. doi:10.1007/978-1-4613-8486-1

[149] Besseling E, Wegner A, Foekema EM, van den Heuvel-Greve MJ, Koelmans AA. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ Sci Technol. 2013;47:593–600. doi:10.1021/es302763x

[150] Wegner A, Besseling E, Foekema EM, Kamermans P, Koelmans AA. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ Toxicol Chem. 2012;31:2490–2497. doi:10.1002/etc.1984

[151] Foekema EM, De Gruijter C, Mergia MT, van Franeker JA, Murk AJ, Koelmans AA. Plastic in North Sea fish. Environ Sci Technol. 2013;47:8818–8824. doi:10.1021/es400931b

[152] Sussarellu R, Suquet M, Thomas Y, Lambert C, Fabioux C, Pernet MEJ, et al. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc Natl Acad Sci USA. 2016;113:2430–2435. doi:10.1073/pnas.1519019113

[153] Leggett W, Deblois E. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Netherlands J Sea Res. 1994;32:119–134

[154] Bailey K, Houde ED. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv Mar Biol. 1989;25:1–83

[155] Fossi MC, Marsili L, Baini M, Giannetti M, Coppola D, Guerranti C, et al. Fin whales and microplastics: the Mediterranean sea and the sea of Cortez scenarios. Environ Pollut. 2016;209:68–78. doi:10.1016/j.envpol.2015.11.022

[156] Rochman CM, Hoh E, Hentschel BT, Kaye S. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: implications for plastic marine debris. Environ Sci Technol. 2013;47:1646–1654. doi:10.1021/es303700s

[157] Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J, et al. Microplastic ingestion by zooplankton. Environ Sci Technol. 2013;47:6646–6655. doi:10.1021/es400663f

[158] Mattsson K, Ekvall MT, Hansson L-A, Linse S, Malmendal A, Cedervall T. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environ Sci Technol. 2015;49:553–561. doi:10.1021/es5053655

[159] Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol Appl Pharmacol. 2001;175:191–199. doi:10.1006/taap.2001.9240

[160] Bhattacharya P, Lin S, Turner J, Ke P. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J Phys Chem. 2010;114:16556–16561

[161] Besseling E, Wang B, Lürling M, Koelmans AA. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ Sci Technol. 2014;48:12336–12343. doi:10.1021/es503001d

[162] Smith GS, Lumsden JH. Review of neutrophil adherence, chemotaxis, phagocytosis and killing. Vet Immunol Immunopathol. 1983;4:177–236

[163] Palić D, Andreasen CB, Frank DE, Menzel BW, Roth JA. Gradient separation and cytochemical characterisation of neutrophils from kidney of fathead minnow (*Pimephales promelas* Rafinesque, 1820). Fish Shellfish Immunol. 2005;18:263–267. doi:10.1016/j.fsi.2004.07.003

[164] Greven A-C, Merk T, Karagöz F, Mohr K, Klapper M, Jovanović B, et al. Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (*Pimephales promelas*). Environ Toxicol Chem. 2016;9999:1–8. doi: 10.1002/etc.15 3501

[165] Rios LM, Moore C, Jones PR. Persistent organic pollutants carried by synthetic polymers in the ocean environment. Mar Pollut Bull. 2007;54:1230–1237. doi:10.1016/j.marpolbul.2007.03.022

[166] Ogata Y, Takada H, Mizukawa K, Hirai H, Iwasa S, Endo S, et al. International Pellet Watch: global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Mar Pollut Bull. 2009;58:1437–1446. doi: 10.1016/j.marpolbul.2009.06.014

[167] Iguchi T, Watanabe H, Katsu Y. Application of ecotoxicogenomics for studying endocrine disruption in vertebrates and invertebrates. Environ Health Perspect. 2005;114:101–105. doi:10.1289/ehp.8061

[168] vom Saal FS, Hughes C. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect. 2005;113:926–933

[169] Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters *in vitro*. Environ Health Perspect. 1997;105:802–811

[170] Fent K, Chew G, Li J, Gomez E. Benzotriazole UV-stabilizers and benzotriazole: antiandrogenic activity *in vitro* and activation of aryl hydrocarbon receptor pathway in zebrafish eleuthero-embryos. Sci Total Environ. 2014;482:125–136. doi:10.1016/j.scitotenv.2014.02.109

[171] Fry DM. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ Health Perspect. 1995;103:165–171

[172] Crisp T, Clegg E, Cooper R, Wood W, Anderson D, Baetcke K, et al. Environmental endocrine disruption: an effects assessment and analysis. Environ Health Perspect. 1998;106:11–56.

[173] Hirai H, Takada H, Ogata Y, Yamashita R, Mizukawa K, Saha M, et al. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. Mar Pollut Bull. 2011;62:1683–1692. doi:10.1016/j.marpolbul.2011.06.004

[174] Holmes LA, Turner A, Thompson RC. Adsorption of trace metals to plastic resin pellets in the marine environment. Environ Pollut. 2012;160:42–48. doi:10.1016/j.envpol.2011.08.052

[175] Boerger CM, Lattin GL, Moore SL, Moore CJ. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar Pollut Bull. 2010;60:2275–2278. doi:10.1016/j.marpolbul.2010.08.007

[176] Davison P, Asch R. Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. Mar Ecol Prog Ser. 2011;432:173–180. doi:10.3354/meps09142

[177] Rochman CM, Hoh E, Kurobe T, Teh SJ. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci Rep. 2013;3:3263. doi:10.1038/srep03263

[178] Yanbin Z, Chen W, Shuang X, Jieqiong J, Rui H, Guanxiang Y, et al. Biosensor medaka for monitoring intersex caused by estrogenic chemicals. Environ Sci Technol. 2014;48:2413–2420. doi:10.1021/es4052796

[179] Rochman CM, Kurobe T, Flores I, Teh SJ. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Sci Total Environ. 2014;493:656–661. doi:10.1016/j.scitotenv.2014.06.051

[180] Zhao Y, Hu J. Development of a molecular biomarker for detecting intersex after exposure of male medaka fish to synthetic estrogen. Environ Toxicol Chem. 2012;31:1765–1773. doi:10.1002/etc.1892

[181] Moore C., Moore S., Leecaster M., Weisberg S. A comparison of plastic and plankton in the North Pacific Central Gyre. Mar Pollut Bull. 2001;42:1297–1300. doi:10.1016/S0025-326X(01)00114-X

[182] Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ Sci Technol. 2008;42:5026–5031. doi:10.1021/es800249a

[183] Anastasopoulou A, Mytilineou C, Smith CJ, Papadopoulou KN. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep-Sea Res Pt I. 2013;74:11–13. doi:10.1016/j.dsr.2012.12.008

[184] Neves D, Sobral P, Ferreira JL, Pereira T. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar Pollut Bull. 2015;101:119–126. doi:10.1016/j.marpolbul.2015.11.008

[185] Romeo T, Consoli P, Castriota L, Andaloro F. An evaluation of resource partitioning between two billfish, *Tetrapturus belone* and *Xiphias gladius*, in the central Mediterranean sea. J Mar Biol Assoc United Kingdom. 2009;89:849. doi:10.1017/S0025315408002087

[186] Bellas J, Martínez-Armental J, Martínez-Cámara A, Besada V, Martínez-Gómez C. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterra-

nean coasts. Mar Pollut Bull. 2016;S0025-326X:30430-30431. doi:10.1016/j.marpolbul.2016.06.026

[187] Lusher AL, Mchugh M, Thompson RC. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar Pollut Bull. 2013;67:94–99. doi:10.1016/j.marpolbul.2012.11.028

[188] Gregory MR. Environmental implications of plastic debris in marine settings--entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. Philos Trans R Soc Lond B Biol Sci. 2009;364:2013–2025. doi:10.1098/rstb.2008.0265

[189] Farrell P, Nelson K. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ Pollut. 2013;177:1–3. doi:10.1016/j.envpol.2013.01.046

[190] Setälä O, Fleming-Lehtinen, V., Lehtiniemi M. Ingestion and transfer of microplastics in the planktonic food web. Environ Pollut. 2014;185:77–83

[191] Smith BR, Blumstein DT. Fitness consequences of personality: a meta-analysis. Behav Ecol. 2007;19:448–455. doi:10.1093/beheco/arm144

[192] Lönnstedt OM, McCormick MI, Meekan MG, Ferrari MCO, Chivers DP. Learn and live: predator experience and feeding history determines prey behaviour and survival. Proc Biol Sci. 2012;279:2091–2098. doi:10.1098/rspb.2011.2516

[193] Lönnstedt OM, Eklöv P. Environmentally relevant concentrations of microplastic particles influence larval fish ecology. Science. 2016;352:1213–1316. doi:10.1126/science.aad8828

Chapter 7

Biotests in Ecotoxicology: Current Practice and Problems

Mariyana Lyubenova and Silvena Boteva

Additional information is available at the end of the chapter

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Abstract

Nowadays ecotoxicology plays the role of a theoretician – methodical unifying centre for the optimization of man – biosphere relations and sustainable existence of life on the Earth. The main basis for its development is the classical toxicology—studies of chemical compounds' effects on man, but ecotoxicology is the original part, following it. According to the modern concept, the ecotoxicology is a science for migration, transformation and utilization of different toxic ingredients (with organic, inorganic or organic-mineral chemical nature; with natural biotic or abiotic origin and artificial, mainly anthropogenic origin) in the environment and their impact on Macro- biological systems with different levels of integration as groups of individuals, population, community, ecosystem, etc. studied in ecology. In this chapter, the types of ecotoxicological tests are discussed in detail with a set of examples about used species, advantages and disadvantages of different types of toxicity testing. The application of exposed natural ecosystems or man-made analogue systems is also commented as the environmentally more realistic approach for ecotoxicological testing. These test systems are increasingly becoming in aquatic ecotoxicology practice, but they are contemporary challenge in terrestrial testing. The development of test systems for realistic assessment of contaminant toxicity is essential for the efficient control of human influence on the environment.

Keywords: ecotoxicology, bio tests, acute, chronic, mono-species, multispecies, biomarkers, kits

1. Introduction: the contemporary meaning of ecotoxicology as a complex science

Ecotoxicology is a scientific discipline, which of the modern stage of man-biosphere relations, is developed as the theoretician – methodical unifying centre for the optimization of these

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relations for the sustainability of life existence on Earth. The main basis for the development of ecotoxicology is classical toxicology—the research of drugs and chemical compounds effects on man. The modern concept of ecotoxicology is that it is the original part following the classical toxicology, which studies migration, transformation, degradation and utilization of toxic ingredients (with organic, inorganic or organic-mineral chemical nature; with natural biotic or abiotic origin and artificial, mainly anthropogenic origin) in the environment and their impact on Macro- biological systems with different levels of integration as groups of individuals, population, community, ecosystem, etc. studied in ecology (MBS) [1, 2] and others.

The main objects of the ecotoxicological studies are the both: (1) toxic ingredients and their "behaviour" in the five main environments such as air, soil, terrestrial, water (incl. sediments) and biotic and (2) the responses of MBS in nature. The studied toxicants can be: (1) by the chemical nature: organic, inorganic or organic-mineral; (2) by the origin: natural (biotic or abiotic) and artificial (mainly anthropogenic origin); (3) by the toxicity: toxicants in the Black list, toxicants in the Grey list, etc. and (4) by the main environment of circulation: air, water, soil or different bio toxicants. The migration, transformation and degradation of toxic ingredients depend on the internal (endogenous) factors, that are, chemical properties of the toxicant and external (exogenous) factors or features of the environment. The studied MBS at different levels of integration can be: diverse individuals as elements of the population; homogeneous and heterogeneous populations as elements of the communities; heterogeneous communities as elements of the bio cenosis; ecosystem as a functional unity between biotope and bio cenosis; landscape, biome and biosphere formed by a corresponding set of ecosystems and their environment. The responses of MBS also depend on the endogenous factors (level of integration and features of the MBS) and exogenous factors (the characteristics of toxicants and habitats). Therefore, according to the used objects, ecotoxicology is an interdisciplinary complex science, developing on the border of chemical, biological, medical, ecological, environmental, economic, social and legal sciences. It can also be considered as applied environmental science studying the biological effects of anthropogenic ingredients. According to the main objects concerned, the main sections of ecotoxicology are *Toxicant dynamics and Bio toxicology.*

Toxicant dynamics considers the migration, transformation and utilization of toxicants according to the characteristics of different environmental areas. There is a great difference in the evaluation of contaminants effects in the laboratory and in the environment. The physical and chemical changes of compounds in the environmental migration lead to the changes of their impact dose that vary in different environments. Therefore, the toxicants in nature often have an indirect effect on biosystems, changing the physical and chemical environment to act upon an indirect effect on the survival of organisms. For the terrestrial environment of great importance to toxicant migration is the temperature-precipitation Dynamics of habitat. In climatology, biogeography and ecology, this dynamics is well characterized by "climatic diagram" ("ombro-thermal" diagram) by Lyubenova [3]. The toxicants emission during the period of drought and semi-drought (calculated on the chart curves) poses an extra risk to the environment, because of the bio systems stress state, limited by temperature – precipitation patterns. The characteristics of different environments and the particular environment are

important for the sensitivity of bio systems to toxicant. The toxicity and behavior of contaminant in the environment are also dependent on the concentration, but also to a large extent, on the predominant form (molecules, ions, complexes, etc.) of migration in biotope and of taken by bio systems.

The development of analytical methods is very important in this division for solving the series of toxicological problems. For example, with the appearance of inductively coupled plasma mass spectrometry (ICP/MS) as a method of measurement, it is possible to separate and determinate the toxicity of various forms of each toxicant. Therefore, the chemical and physical measurements for assessing the toxicity of the substances and their forms are important for determining the valid toxic concentration in the bioassay and complementary test system reply.

Bio toxicology examines the effects of toxicants on sensitive test systems and further on MBS in nature. For the manifestation of this effects is necessary to consider four main phases in passing of toxicants in bio systems, namely biological absorption and assimilation, metabolism, transport and excretion from the system. At the MBS level, biological uptake and accumulation from the environment (bioaccumulation) in the food chains (biomagnification) and accumulation in different organs or elements of bio system (bioconcentration) can be characterized by calculating several geochemical coefficients [4]: biological absorption coefficient (BAC); relative absorption coefficient, (RAC); acropetal coefficient (AC); temporal absorption coefficient (TAC); litter-mulch coefficient, A; and relative rate of transformation of the organic matter (RRTOM).

There are few main differences between classical toxicology and ecotoxicology: (a) the usage of bio-test, including selected for this purpose; (b) the main objects for acute toxicity measurement are different – *Daphnia spp*., or laboratory rat (c) the reference of induced toxicological effects on sensitive/representative test objects to MBS in nature; (d) the usage of standardized methods and indicators (good laboratory practice) guaranteed the results recognition everywhere, etc. The requirements for the test bio systems are: susceptibility of cultivation and maintenance in laboratory, low-cost, highly responsiveness to the toxicant, mass usage and vast database available, representativeness of the exposed species to MBS, low correlation with other assessments in the same trophic level and strong correlation with a series of changes in ecosystems [1]. Today, in laboratory tests with test objects, most often are reported mortality, reproductive capacity, changes in growth, development, behaviour, biochemical, genetic changes and other.

The MBS are characterized by a complex structural and functional organization and the specificity of the set of internal regulatory mechanisms that support the system in equilibrium, which should be considered in the toxicological effect extrapolation, as well as for assessment of ecosystem health and predicting the risk. Therefore, the models, adequately reflecting the responses of MBS in nature, require the knowledge of structure, function and the mechanisms for ensuring the existence and integrity of MBS and the behaviour of toxicants in the current climate. The main functions and features of MBS have been deeply commented by Lyubenova [5].

The main aim of chapter is to comment the contemporary knowledge and established practice in the usage of bioassays to study the environmental toxicity of ingredients. The acute, chronic, mono-species and multi-species tests are discussed. Moreover, the analytical and biochemical methods for determining the initial damage at the molecular level on acute and chronic exposure are commented, too. The molecular markers (biomarkers) or indicators are very important for the early diagnosis of damages and the interests for new developments are growing steadily.

2. Ecotoxicological testing: contemporary knowledge and gaps

The ecotoxicological effects of contaminants on bio systems and MBS are developed as sub lethal and lethal responses. The earliest toxicological responses (change in biological systems) are detected at the cellular level. Some of the most important effects are changes in the structural components of the cell membrane (e.g. breach links between proteins and lipids); suppression of certain enzymes (e.g. microsomal enzymes); damage to the whole or partial metabolic changes (e.g. the synthesis of carbohydrates) [6]; changes in DNA correlation, respectively mutations and modification of cell growth [7], etc. At the macro-bio system level, effects related with their structure and functioning can be observed: efficiency of energy utilization and transmission through the food chains [8]; bio-depressant effects (inhibition of growth and reproduction) [9] and bio-stimulant effects (e.g. eutrophication) on population or community [10]; changes in the nature of biological cycle—capacity (the amount of chemical elements involved in biomass per year), intensity (of productive processes, energy transformation and destructive processes), chemistry (determined by the leading elements in the cycle), openness (e.g. balance of import and export); bioaccumulation of toxicants (higher concentration in the biomass than in the surrounding medium) [11]; bio magnification (increasing the concentrations in each higher-trophic level) [12]; bio concentration (accumulation in separate organs or elements of bio-system), etc. In most cases, plants and animals are more exposed to the combined effects of many pollutants simultaneously [13]. The interaction between them may increase or decrease the toxicity of the mixture and hence alter the response of the biological system. The effects on biosystem exposure on two or more toxicants may result in the following combining toxicological responses: supplementary response (e.g. in simultaneous action of two organophosphate compounds) [14]; synergistic (reinforced) response (e.g. response of rat to concomitant ingestion of hepatic toxins – ethanol and carbon tetrachloride) and depressed (antagonistic, reduced) response, when the antagonistic reaction between toxicants exists: for example, chemical (e.g. the toxic effect of Se and Hg) [15], competitive (e.g. the toxic effect of CO), uncompetitive (e.g. the toxic effect of atropine and organophosphorus insecticides), functional (e.g. the toxic effect of barbiturate decreased vascular pressure) and predisposing (e.g. the reduction of organophosphorus insecticides toxicity with piperonyl bioksid by blocking the activity of cytochrome P450, responsible for the metabolism of organophosphates) antagonism. The interactions between toxicants and natural chemicals in the environment result in formation of new molecules or complexes, changing their expected utilization. When toxicity is unknown, the conducting tests use a wide range of concentrations

and report of "all or none" response. The dose–response relationship is the tested biological effects to 4–5 toxicant concentrations that cause from 20 to 80% mortality. This value is only representative of acute exposure, not chronic one. The LD_{50} and ED_{50} variables are influenced by many factors: behaviour of animals [16], age [17], sex [18], temperature [19], water quality (hardness) [20], pH [21], etc. Nevertheless, the 50% response rate is used, because it is the most reproducible response and can be calculated with high reliability. There are three main types of systems for the contaminants exposure of aquatic organisms: *Flowing-through, Static and Renewal* [1 and others]. Flowing-through systems are preferred for the study of acute toxicity. They are recommended for toxicants with high volatility and pollutants that are unsustainable in water [22 and others]. The static systems are applied mainly in short-term tests (≤96 h with fixed or slowly degradable materials and at a low load (biomass/water volume) of the test organisms. Static systems are used at limited availability of studied pollutant critical load with residual water or receiving toxic effects at critical levels of the components of the test. The renewal systems are applicable in work with small organisms that may be lost in watercourse systems or that are very sensitive to streams. They are also used in the case of limited test material. A *recirculation test* is similar to a static test except that the test solutions and control water are pumped through an apparatus, such as filter, to maintain water quality but not reduce the concentration of test material. The water is returned to the test chamber. This type of test is not routinely used because of uncertainty about the effect of the apparatus (aerator, filter and sterilizer) on the test material [1].

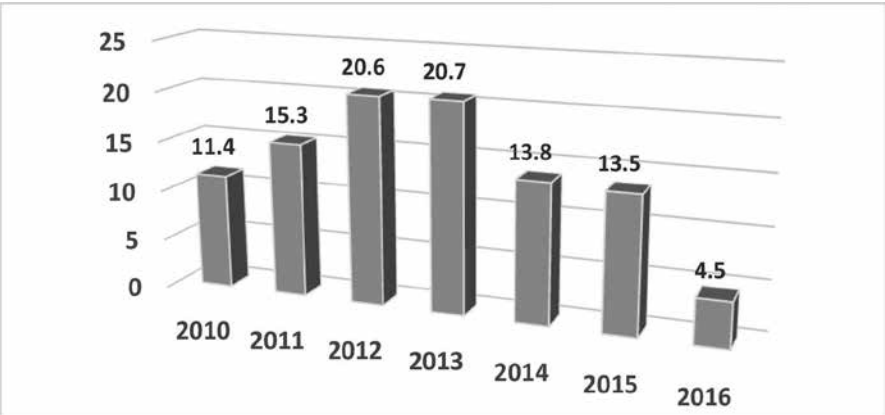

Figure 1. Percentage distribution of ecotoxicological studies published based on random sampling.

In the random sample of 384 published studies, 535-conducted bio-tests were considered. For the studied period (2010–2016), an exponential increase of published ecotoxicological studies to 2012 can be observed—**Figure 1**. The level is kept in 2013 and the percentage sharp fell in 2014, while in 2015 the published ecotoxicological studies are closed to that in 2014. We do not have compete data for 2016, but it seems that this trend will likely keep. Furthermore, the scientific community is concerned about finding new environmentally acceptable agents and technologies in industry, agriculture and households, which is gradually becoming a priority

in the new solutions. No less is the role of environmental legislation, the timely testing of new toxicants and the introduction of regulations and restrictions.

Among the reviewed studies, the tests for toxicity of aquatic environment prevail – 67%, of which these for the toxicity of freshwater are 35%, the terrestrial ones are about 20% and those concerning three environments – 13% (**Figure 2**).

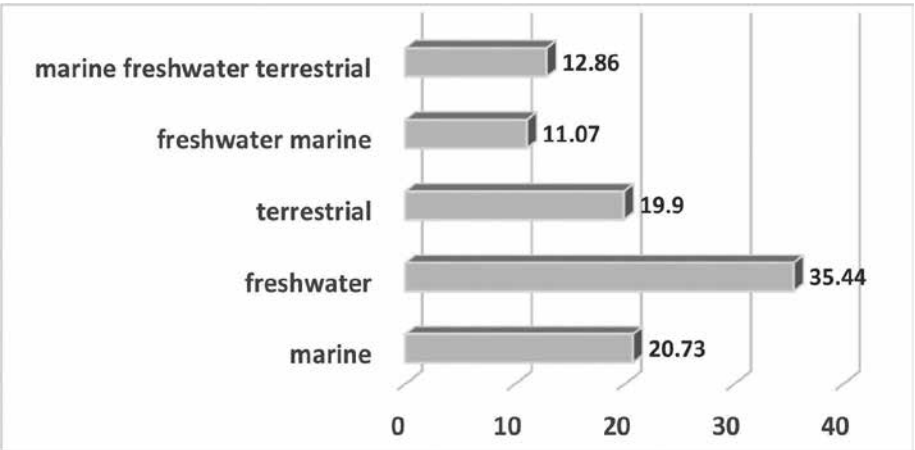

Figure 2. Percentage distribution of ecotoxicological studies by media published in random sample.

During the years, the focus of researches has been on the toxicity of different environments, for example, in 2011, prevailed these for the aquatic environment; in 2012, for the terrestrial; in 2013, again for the aquatic; and in 2015, again for the terrestrial (**Figure 3**).

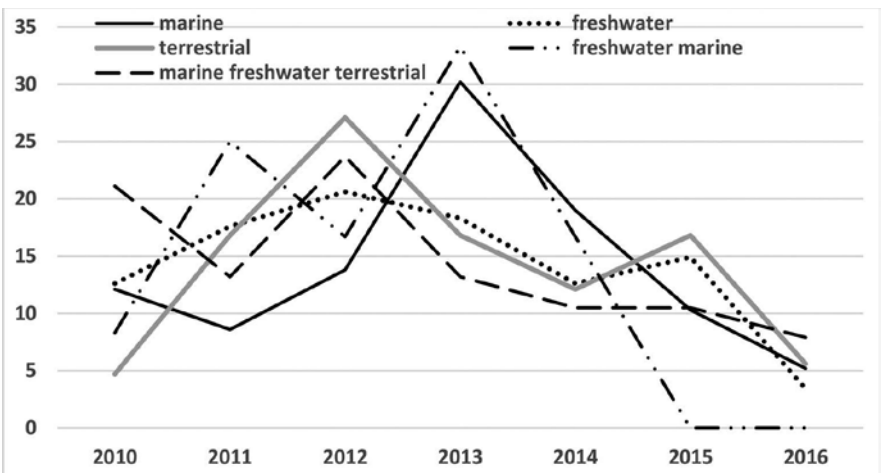

Figure 3. Percentage distribution of ecotoxicological studies by media and years published in random sample.

For the research period in the toxicological testing, 25 groups of biological systems have been used as most tests have been performed with crustaceans and fishes, 22% and 20%, respectively (**Figure 4**). Common test objects are also insects (9%), molluscs (9%), algae (8%) and the plants (6%). In the ecotoxicological studies, 61 crustacean species, 51 fish species, 27–17 insect species, molluscs, algae and higher plants have been used (**Figure 5**).

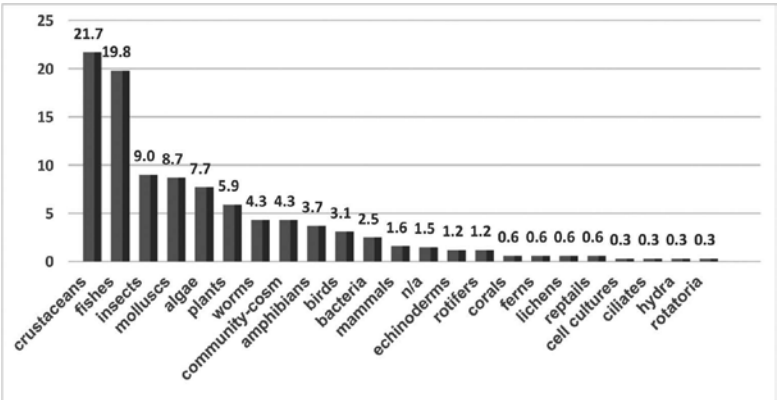

Figure 4. Percentage participation of biological groups for ecotoxicological testing in random sample (2010–2016).

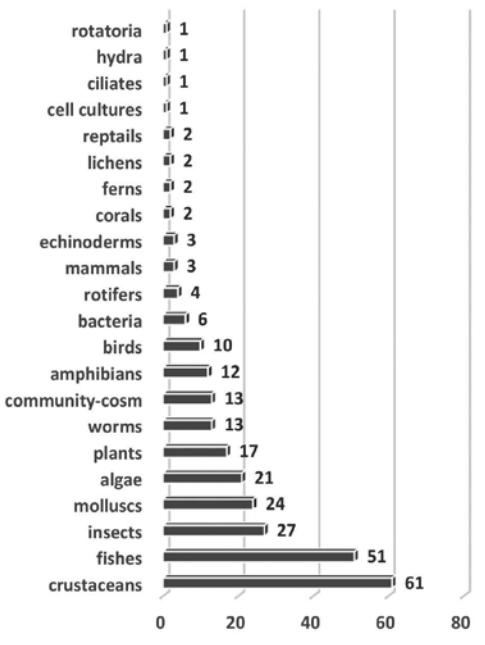

Figure 5. Participation of biological groups (number of species) for ecotoxicological testing in random sample (2010–2016).

2.1. Ecotoxicological tests for short–term (acute) and continuous (long–term,6 chronic) toxicity

The accepted types of toxicants impact on exposed biosystem are: acute (high doses and for a short time, typically 24 – 96 h); sub-acute (repeated exposure for one month or less at lower doses than those in the acute exposure); sub chronic (multiple exposure, for 1–3 months) and chronic (exposure for more than 3 months at doses representing about 1/100 to 1/1000 of the acute dose). The exposure intervals definition varies for different biological systems, media and toxicants.

For the period concerned, the studies of acute toxicity clearly prevailed over the chronic tests. In a number of studies for clarifying the actual toxicity, a series of both tests have been performed. The practical application of subacute and sub-chronic tests is low or negligible (**Figure 6**), but the trend of increasing the interest for these tests in 2014 and 2015 is noticeable.

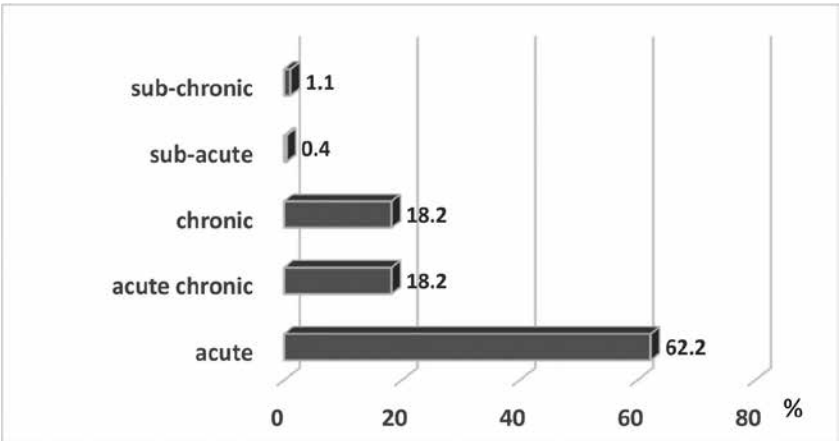

Figure 6. Percentage participation of acute and chronic tests in random sample (2010–2016).

Acute ecotoxicological testing has two main applications in environmental risk assessment. One of the applications is in conducting the screen test, for example, to determine whether the toxicant is biologically active at test doses for used indicators. The second type of application is the determination of acute toxicity—measuring the dose-response function and determination of LC_{50}/LE_{50} for a predetermined period. The acute toxicity tests is the first step for detecting the total toxic effects caused by toxicant. Many studies have been dedicated for searching to the most sensitive species to conduct acute tests. The practice shows that there are no universal species, that are the selection of species depends on the type of toxicant and the affected ecosystems. Virtually every hydrobiont is suitable for conducting the acute tests, but one of the most used are *Daphnia magna* [23–26], *Pseudokirchneriella subcapitata* [27, 28]; many mussel species like *Dreissena polymorpha* [7] and *Mytilus edulis* [29]. Fish species are also very often used as test system: *Danio rerio* [30, 31], *Gambusia holbrooki* [32], *Cyprinus carpio* [33, 34], *Oreochromis niloticus*s [35, 36]. The terrestrial test objects include bees [37, 38], *Mus musculus* [39], *Megascops asio* [40], *Podarcis sicula* [41] *Aquila adalberti*, soil invertebrates [42] and other.

The bacterial species, especially *Vibrio fischeri*, are also used in many ecotoxicological tests [27, 28, 43] and others. Today the most used plant test objects are different species of algae [44] and duckweed (*Lemna* spp.) [45]. They have a high reproductive capacity and therefore the study includes several generations in a relatively short period. Intensity of photosynthesis [46] and growth [47] are measured at algae or growth [48] at duckweed. Reporting indicators for the photosynthesis intensity are: concentration and ratio of pigments in photosynthetic biomass [49], amount of released oxygen [50], assimilated $14CO_2$ [51], ATP production [52] and number of cells [53]. Short-term sublethal tests are used to evaluate the toxicity of effluents to aquatic organisms [54], but some authors use terrestrial organisms [38]. These methods are developed by the EPA, and only focus on the most sensitive life stages. The endpoints for these tests include changes in growth, reproduction and survival as NOECs, LOECs, LC50s and EC50s [25] and others. Acute toxicity resulted in abortion rate of eggs and embryonic stages [25], reduced offspring and egg production [55], reduction in hatching success [56], decrease in fecundity [57], decrease in fertility [57], failure of metamorphosis [58], delayed development [59] and abnormalities and deformities in fish larvae [60]. Despite the results cited above, [61] reported no significant effects of the insecticide indoxacarb on the eggs, young and old larval stages and the pupal stage of two species of Trichogramma [62] and observed no negative effects on *Daphnia magna* embryonic development or hatching rate to insecticide Carbaryl up to concentrations almost 1000 times of the median effect concentration (EC_{50}) of neonate survival in acute tests. Furthermore, [63] suggested that adaptation to tolerate PCBs has altered the sensitivity of *Fundulus heteroclitus* to oxidative stress during embryonic development, demonstrating a cost of the PCB resistance adaptation and [64] reported resistance of *Fundulus heteroclitus* from the Atlantic Wood Superfund site on the Elizabeth River to the acute toxicity and teratogenesis caused by polycyclic aromatic hydrocarbons (PAHs) and others.

Chronic ecotoxicological testing is subjected to determine whether the long-term toxicant exposure that is supposed to be present in the environment, can have a significant detrimental impact on ecosystems. The number of selected species that inhabit the ecosystem is tested to toxicant exposure. The threshold of chronic concentration complies with the reactions of the most sensitive species. In the practice, the following assumptions are made to the chronic toxicity determination: selected individuals are with respective sensitivity to toxicant corresponding to the representative natural groups; chronic threshold concentration set for the most sensitive species, is the starting chronic toxicity for the ecosystem; studied species are the most sensitive to the toxicant in the ecosystem. The chronic tests provide information allowing extrapolation of the effects at the community and ecosystem level [1]. In the chronic tests, the bio-reactions upon exposure to toxicants for a long time are examined [65], often as long as the entire life cycle. After running the assay, the established initial concentration causing chronic sensitivity of the ecosystem is compared with the expected concentration of toxicant in the environment. Effect of severe chronic toxicity can be expected at concentrations exceeding the established in the environment initial or threshold concentrations. For the predicting of toxicants chronic effects, commonly three categories of tests are used: including life cycle [66], including the most sensitive life stages [67] and functional. By the lifecycle tests, the contaminant impact of chronic exposure on reproduction, growth, survival and other indicators of several generations of test organisms are examined. The tests begin with eggs, larvae or

juveniles and continue until the reproduction of test organisms. The used toxicant concentrations range from causing strong negative impact to at least one that has no influence on studied indicators (compared to controls). Most commonly aquatic organisms that can complete their life cycle in laboratory are used—algae, invertebrates and others. In these tests, the calculation of survival and fertility by age is conducted according to equations of Lotka or Yuler [1]. The isolated generations in the period of youth and the period of maturity may be also used for these tests. The toxicity tests on the life cycle require considerable time and costs, especially for vertebrates. The tests conducted on the most sensitive life stages are used for studying the contaminants impact of chronic exposure to the survival and growth of eggs [68] and larvae [69] of fish. The indicative tests have been developed with the early – eggs floating on the water surface or eggs laid on the bottom of rivers or estuaries, embryos or larvae. In surface water micro-layer are concentrated heavy metals, detergents, chlorinated oil hydrocarbons, etc. The sediments also accumulate a number of toxicants. The tests start with the exposure of groups of fertilized eggs or embryos through the system for supply of serial concentrations of tested toxicant. The range of concentrations should include substantial effects and lack of impact. The species inhabiting cold water, for example *Salmo gairdneri*, are usually exposed for a period of 330–570 days [1] while inhabitants of warm water, for example zebrafish, are exposed from 30 to 250 days [70]. The parameters of the measurement include survival, growth and teratogenesis. The benefits of testing embryos and larvae are: saving time and money; creating opportunities for the study of larger number of species compared with the life cycle tests; calculated thresholds for chronic toxicity can be extrapolated to many more species for a wide range of areas and trophic levels than the potential in the implementation of testing lifecycle; the needs to conduct these tests due to the insufficient data on the fish toxicity. The concentration of toxicant that causes chronic toxicity effects on eggs, embryos and larvae vary among the same species and among different species. It depends on the duration of the conducted test—stage of the life cycle or the whole life cycle. For example, the studies with small *Salmo gairdneri* fish has the highest degree of sensitivity to six toxicants, while the eggs are relatively resistant, because of the bio–absorption alteration. The early life-staged tests are not considered as valid, if mortality in the control sample is greater than 30% [1]. Some authors published results of conducting tests with eggs [71], fish embryos [28], larvae [72] and early stages of development [24], as through them the potential toxic effect is reflected.

By the *functional tests,* the effects of toxicants on various physiological functions of test objects are studied. The fishes and other aquatic organisms react physiologically and with behavioural changes on toxic exposures. For example, changes are observed in blood chemistry [73], enzymatic activity [74], histology [75], swimming behaviour [76], sensory perception and disease resistance. This testing has some disadvantages: the effect of adaptation to toxicant is absent and the reported effects differ from the real ones; the inability to capture all variation in functional parameter for MBS; the inability to extrapolate the results to MBS. In general, the information is about the relationship between functional individual bio-effects to toxicants and the survival, growth and reproductive capacity of the populations in community. The data for the discussed test categories are used to determine the threshold concentration of the toxicant causing chronic toxicity.

Bioaccumulation tests are toxicity tests that can be used for hydrophobic chemicals that may accumulate in the fatty tissue of organisms. The toxicants with low water solubility generally can be stored in the fatty tissue due to the high lipid content in this tissue. The storage of these toxicants within the organism may lead to cumulative toxicity. Some authors report results from bioaccumulation in different tissues and organs like liver, kidney, gills, embryo tissue and accessory glands [60, 77]. Bioaccumulation tests use bio concentration factors (BCF) to predict concentrations of hydrophobic contaminants in organisms [78] and others. The BCF is the ratio of the average concentration of test chemical accumulated in the tissue of the test organism (under steady state conditions) to the average measured concentration in the water.

Tests with sediments. At some point, most chemicals and elements originating from both anthropogenic [79] and natural sources [80] accumulate in sediments. For this reason, sediment toxicity can play a major role in the adverse biological effects seen in aquatic organisms, especially those inhabiting benthic habitats [81]. A recommended approach for sediment testing is to apply the Sediment Quality Triad (SQT), which involves simultaneously examining sediment chemistry, toxicity, and field alterations so that more complete information can be gathered [82]. Collection, handling and storage of sediment can have an effect on bioavailability and for this reason standard methods have been developed to suit this purpose [83]. Some ecotoxicological tests for assessing sediments quality are published [84]. The worms [42], clams [85], fish [86] and phytoplankton [87] are mostly used as test objects.

2.2. Mono–species tests and multi–species tests

For the period concerned, the studies of acute toxicity clearly prevailed over the chronic tests. In a number of studies to clarify the actual toxicity the series of both tests have also been performed. The practical application of subacute and sub-chronic tests is low or negligible (**Figure 6**), but the noticeable trend of increasing the interest for these tests in 2014 and 2015 is observed (**Figure 7**). The analyses carried show that in 2012, 2014 and 2015, the tests with two species as test objects and multi-species ones were applied in most published studies. In 2013, the focus is mainly on tests with communities and multi-species ones.

Mono-species tests are appropriate in determining the toxicological effects on individual characteristics of species such as mortality [88], growth [89], reproductive capacity [41], behaviour [38] and other but have limited significance for the consequences on the entire ecosystem from the pollutants impact [36]. The disadvantages of mono-species testing are: the responses of individuals are often not sufficient to extrapolate responses of other (even very close) species and determine the real toxical effects in nature; the identifying of sensitive species or groups to the toxicant is expensive; the influence of indirect effects from intra-population and inter-population relationships on toxicity cannot be observed; the standardized laboratory conditions in conducting mono-species tests are different from the conditions in the biotope [90]. The influence of many additional abiotic and biotic factors is always present in the field effects changing significantly the eco–toxicological response. The mono–species tests have been used for years for the simulation of multi-species effects in ecosystem although the existing inadequacy. Therefore, the results obtained in mono-species tests are more often used in practice and have the same rank of importance to those of multispecies tests enable to assess

indirect effects, such as the changes of structure and the functioning of ecosystem (changing in competition, predation, energy flow and circulation of substances, etc.).

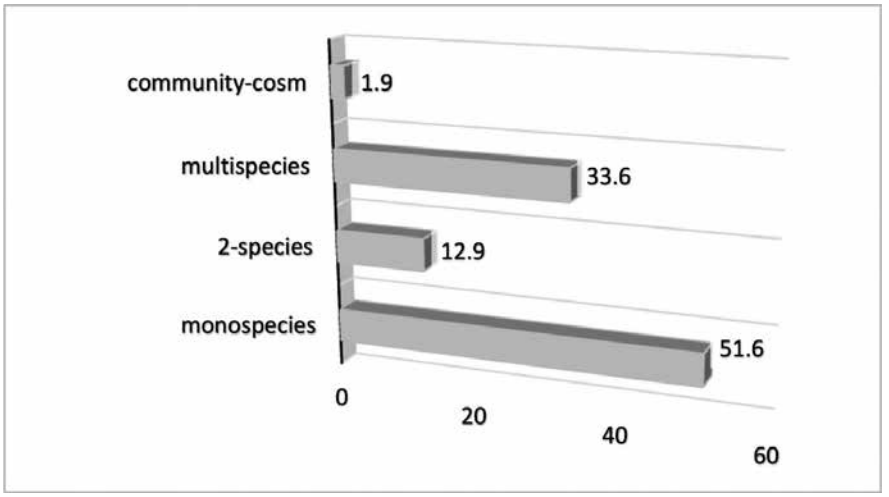

Figure 7. Percentage participation of tests with different number of test objects used in random sample (2010–2016).

Multi-species tests. At every level of biological organization appear new features that could not be recognized even by the most intense and careful studies of low levels. The complexity of MBS makes unacceptable the assumption that the responses received in toxicity tests of various species are applicable for predicting the behaviour of system of integrated species [91]. Therefore, the biological effects within the natural system differ considerably from those in laboratory tests [1]. Only acute toxic effects can be accounted with available methods. Another way to resolve the testing problems is by the identification of sensitive or/and key species or groups of species for MBS, but it is very expensive approach. We also need to bear in mind that in multispecies tests many answers are skipped due to unification of indicators, individuality of responses and statistical unreliability of additional indicators. So, we are faced with the inability to reproduce the experimental results to MBS level due to the variability and specifics of relationships, self-organization and self-regulation of different MBS. In other words, the uniqueness of each MBS leads to the inability for the toxicant assessment unification. The toxic effects extrapolation to ecosystem level requires good description of the biotope conditions and availability of data on the structure and functional processes. The lack of classification and characterization of ecosystem types in relation to specific environmental factors and bio-system features hampers the ecotoxicological research interpretation. For example, the population generally shows a lower sensitivity to pollutants than the individuals do. It seems that environmentally more realistic approach includes monitoring the effects of exposure to toxic impact in natural ecosystems or man-made systems specially designed to resemble fully the characteristics of natural systems (multi-species tests with the key representatives of studied ecosystem – model ecosystems, microcosm, mesocosm, lake-coral systems and others). The most optimal combination of test objects for aquatic ecosystems

includes species of algae, crustaceans and fish, reflecting their functional structure. The micro-ecosystem is not an absolute analogue of the natural ecosystem, but a small system with sufficient complexity that could allow realistic ecological study of the typical characteristics of ecosystem in nature. Their application to environmental toxicology has been of interest, where fate and behaviour of contaminants markedly modify the exposure of biota to them and hence the environmental hazard [1]. In the aquatic micro-cosmology, there are two major achievements even in the last century: the ecosystem model and the food chain model. The ecosystem model is used to study the inherent ecosystem properties and functions in the level of integration—cycling of substances, energy flow and homeostasis. The food chains model is applied in studying the relationships predator-prey, environmental efficiency in the transmission of energy in trophic levels, etc. The experiments with food chains model is simpler and easier to manage compared to experiments with ecosystem model. The main problem is in selecting the appropriate microcosm types for different studies. Many species tests include a set of standard reproductive tests on daphnia, where the dose–response relationship is determined depending on the supply of nutrients [1]; large – (CEPEX, 1300 m^3); medium – (10–150 m^3) and small-sized (1.4 m^3) bags or tanks is used for the isolation of communities living in the open sea. Usually the larger the system is, the longer it will operate and the environmental conditions, community structure and functioning will look more like natural. The artificial macro-bio-systems differ by the nature of communities; by the period of submission of the matter—long and short duration, also by using materially closed systems. The classical example is Taub test [1]. He performed to some degree a standardized toxicity test with 24 identical 3.6 1 containers that are "infected" with a total of 10 algal species, five animal species and unknown set of bacteria. The duration of the experiment was 60 days due to deterioration of the community in the absence of normal biological cycle. In Kersting classical test [1], the lack of mineral circle was overcome. He has developed a microcosm with 151 Compartments, in which primary production, secondary production and decomposition are separated to prevent overconsumption. The balance between production and decomposition in this type of microcosm proves its sustainability for months or even years in relatively stable conditions, which corresponds to the "ecological temporal periods". These types of models that are designed to resemble certain types of natural systems (isomorphic models) usually include sedimentary part and benthic invertebrates and operate under watercourse conditions. In most cases, it is expected the water, sediments and biota to be modelled from the site. There are designed different tanks in size (560 1 or 13 m^3) with a variable depth to external structures that are best described as model flows, model channels and model lakes of various sizes. These test systems can combine the evaluation of biological effects with studies on transformation of pollutants in the environment. Test systems modelling the changes of interactions between populations under the chemical stress were also developed in the last century. The tests of Cairns and Lundgren [1], studying the interactions of algae and daphnia populations under chemical stress, are well known [1]. The usage of a battery of bioassays involving different species at different trophic levels is an efficient and essential tool for predicting environmental hazards to the aquatic ecosystem. For example [85], the adverse effects of wastewater treatment plants (WWTPs) on sediment quality at the Bay of Cádiz (SW, Spain) were investigated using a battery of acute bioassays and chemical contamination. The author concluded that the test may provide

complementary information for diagnose of environmental factors that can impair aquatic communities. In other study [92], a battery of marine and freshwater species representing different trophic levels was used, and compared the bioassay of sensitivity levels to pharmaceutical residues of three antidepressants (fluoxetine, sertraline and clomipramine). The bioassays like the algal growth inhibition test (*Skeletonema marinoi* and *Pseudokirchneriella subcapitata*), the micro-crustacean immobilization test (*Artemia salina* and *Daphnia magna*), development and adult survival tests on *Hydra attenuata*, embryotoxicity and metamorphosis tests on *Crassostrea gigas*, and in vitro assays on primary cultures of *Haliotis tuberculata* hemocytes were selected. The importance of test battery usage showing the difference in sensitivity between bioassays hence high interspecies variability in EC50–values was underlined. The battery of bioassays and representative aquatic organisms (*Vibrio fischeri*, microalgae *Pseudokirchneriella subcapitata* and invertebrate *Daphnia magna*) for assessing the acute toxicity of water-extractable fractions of biochar-amended soil was published [93]. Based on the obtained results, the authors suggested that the battery of rapid and cost-effective aquatic bioassays that account for ecological representation can complement analytical characterization of biochar-amended soils and risk assessment approaches for surface and groundwater protection. The battery of bioassays was used [94] to assess the impact along a river due to a leak of effluent from an Installation of Cleansing and Uranium Recovery (Tricastin, France) and provided an estimation of exposure conditions that occurred along the river. The acute toxicity of the effluent was evaluated and compared to the toxicity of uranium nitrate in bioassays using *Chlamydomonas reinhardtii*, *Daphnia magna*, *Chironomus riparius* and *Danio rerio*.

The dysfunction occurring in plant communities and its effects on the plant populations structure and functioning is very well studied, especially for systems with poor species composition and simple structure. The pioneer studies have been published [1]. The imbalance is defined as a sudden change of the resource base that led to a clearly visible response. The hierarchical series of responses to these impacts can be predicted. The responses of the plant may influence the plant populations—reproduction, density spatial structure, rate of growth, mortality, body age, genetic variations, interspecies connections, etc. The different responses of plant populations can lead to major change of plant community as species composition, species richness, distribution of included genera, succession processes, etc. At the ecosystem level, these changes affect the primary productivity, the intensity of respiration, the intensity of mineralization and other functional processes. These responses depend on the type, frequency, intensity, duration and heterogeneity of dysfunctions. In some cases, we can evaluate the different obtained effects to the intensity and combination of impacts in models of vegetation structure and dynamics changes. By the comparison between the responses of exposed plant communities and the responses of untreated ones that grow on compatible soil types under similar topography and climate, the imbalance can be evaluated. Further, it would be possible to find a correlation of the results from laboratory tests, such as root growth, the growth of algae in soils or soil extracts, with actual plant data. There are some attempts to create models and study the complex toxicity on terrestrial ecosystems, but they are mostly with cultural ecosystems. For example, a model toxicological investigation of cultural plant-soil complex treated with wastewater have been published [95, 96]. Today, the conducting ecotoxicological studies with model ecosystems are common practice in the aquatic toxicology.

While it can be considered that to some degree the problems associated with the study of the toxicological effects in the aquatic toxicology are resolved, this is not the case in terrestrial toxicology.

Tests with nanoparticles Nanoparticles represented by a group of toxicants as TiO_2 [97], ZnO [44], carbon nanomaterials (CNMs) [98], core-shell copper oxide [99], silver (AgNPs) [49] and others are tested in ecotoxicology. Mainly, tests with aquatic organisms are conducted like algae [97–99], plants and invertebrates [97].

Biomarkers The most used biomarkers is the activity of antioxidant enzymes like catalase (CAT) [100], superoxide dismutase (SOD) [101], glutathione S-transferase (GST) [102], glutathione peroxidase (GPX) [101] and glutathione reductase (GR) [101] or their gene expression. Many authors use Hsp70 (heat shock proteins) and their expression for determining the toxicity of a pollutant [102, 103]. When using fish as test object, the haematological parameters are often applied as biomarkers [104]. Behavioural biomarkers are applied for frog tadpoles [105], clams [106]; fish [31], cladocerans [107]. When using plants as test object, cell viability (mitochondrial activity) and plant physiology (chlorophyll) are used as biomarkers [108].

3. About the studies of ecosystem health (ecosystem diagnosis)

The evaluation methods for ecosystems health assessment are usually based either on *risk assessment* or on *bio assessment* [1]. Most studies attach importance of *risk assessment*, but it is appropriate when the effect caused by known toxicant from one or more known sources with relatively high emissions and expected acute effect. The risk assessment focuses on the chemical composition, the impact of environmental toxicity and laboratory data. The acute tests with test object definitions as well as the available kits usually are used. The data of plants and soil invertebrates can be used to study the response of the short exposure, especially when impacts were made at regular intervals. The upper layer of the soil (5 cm) and plants (root, leaf and stem) are collected for the acute toxicity testing. It is essential also to measure physiological parameters—respiration, photosynthesis, pigments as well as microbial communities indexes. The short response of soil microbial community is also suitable indicator for this review—for example, the intensity of soil respiration of exposed and unexposed soil. The studies about the ecosystem's risk assessment using GIS, aerospace technologies and calculation of State Vector also were published [109–113 and others].

Bio assessment is applied for the complex effects of mixed toxicants or for low non-specific toxicity similar to chronic effects. The assessment focuses on ecosystem characteristics, factors causing stress and their importance and usage of measurements and models for chronic effects assessment. Bio assessment includes micro- and macro-research to perform controlled tests for ecosystem under impacts. It is necessary to know the characteristics of studied ecosystems and principles of their self-management and self-control. The bio-assessment of ecosystem state is based on the results of tests series with "critical" ecosystem components for chemical, physical and biological effects on ecosystems. There have been published 44 different characteristics important for the bio-assessment of ecosystem state and the eight of them are identified

as critical ones of varying importance for different ecosystems. These ecosystem characteristics are as follows: (1) The features of the habitat to maintain biodiversity and reproduction of organisms; (2) The phenotypic and genotypic diversity of organisms; (3) The length of food chain, supported by biotope; (4) The determining biological production level of active and stored nutrients in biotope; (5) The features of biological turn-over for maintaining the ecosystem existence; (6) The energy flux to maintain the trophic structure; (7) The set of feedbacks for self-regulation and (8) The capacity to regulate the toxic effects, including capacity for biological absorption, metabolism and decomposition of toxicant, linking with the anthropogenic influences buffering. The importance of every critical characteristic depends on the ecosystem geographical location (i.e. eco region) and whether the system is aquatic or terrestrial. Some authors published bio assessments for different ecosystems, by selection of a set of indexes for the noticed critical characteristics [114–117]. The complex of 44 characteristics, even the complex of eight of them, the variation in the relative importance of each of them for the different ecosystems and the lack of standardized indices and methods for each of them, makes the representative assessment unlikely at each case. The representative assessment of ecosystem can only be done with a few "standard" test systems types. Today the scientific community makes efforts to resolve these methodological problems, not only in relation to ecosystem diagnosis, but also to assess the ecosystem capacity, assets and services [118].

The need of test systems classification leads to the publication of a set of investigations in the last century. For example, the classification [1] is based on the following criteria: environment (air, water and soil); time of exposure (long, medium, occasionally, etc.); concentration of toxicant (mg/l, mg/m^3); used organisms (bacteria, fish, mammals, plants, etc.); type of exposure (through food, air, dermal, etc.); the effects on test objects (genetic, toxic, bio-accumulation, etc.); measuring methods; test type (common, standard, experienced, screening); requirements for variability, accuracy and precision of values and technical requirements for personnel and laboratory equipment. The developed protocols, however, require significant modifications depending on the type of ecosystem and environmental factors, the objectives of the study and more mentioned in the specific dynamic action analyses (SDA). To assess the possible effects on ecosystem level, the responses of dominant species are usually investigated on a set of sample plots. The mostly used indicators are grouped as indicators of plant community, plant chemistry (major cations, nitrogen, phosphorus, iron, zinc, etc.), aboveground and water insects (some populations of *Homeoptera* are extremely sensitive to changes in the chemical and species composition of communities), soil invertebrates, soil chemistry (indicators for mineralization potential of organic nitrogen and phosphorus, variable cations, pH, inorganic nitrogen, total nitrogen, organic and inorganic phosphorus), water chemistry, organic matter decomposition (the activity of heterotrophic microorganisms and species of class Arthropoda, etc. [119, 120 and others]. A lot of plant community indicators and indices are applied at ecosystem level as plant species composition and density (number/m^2); plant biomass, separated into herbaceous and woody, above- and underground, live and dead; the average height of the stems; specific leaf mass (SLM); stalk weight; total dry weight (W); total leaf area (Le), etc. The relative indexes are also calculated as: the relative growth [$Rw = (1/W)\cdot(dW/dT$]; relative leaf area [$Re = (1/La)\cdot(dLa/dT)$]; leaf ratio [$F = La/W$]; full leaf evaluation [$E = (1/La)\cdot(dW/dT)$] and others. Soil invertebrates as earthworms, spiders and nematodes are sufficiently

sensitive to the quantity and quality of plant roots and plant detritus and can influence or be influenced by microbial populations. This group of consumers is identified as the potential regulator of the decomposition and production processes. The macro arthropods and earthworms are associated with the fragmentation of detritus, while the micro arthropods and nematodes are more important for the micro-organisms populations. Earthworms are a standard system for the bio assessment. The soil nematodes and the species of *Scarabaeidae* family are linked to net primary production and are sensitive to changes in plant chemistry. The number and trophic composition of soil invertebrates are measured together with the biomass of plant roots and rhizomes and dead biomass. The plant carbon distribution changes in stress are also a good indicator for toxicity. The data for a living and dead biomass in relation with the number and composition of soil invertebrates provides possibility for ecosystem state assessment. Usually the number of earthworms and large arthropods is measured in spring and autumn using soil samples of 0.1 m^2 and 30 cm depth. The micro arthropods and nematodes are measured by mechanical separation of soil samples.

For the forest ecosystem health assessment, the widespread indicator is defoliation that can do possible to calculate the ratio of damage of ecosystem: $C = [\sum(n \cdot k)/NK] \cdot 100$, where n is number of trees with respective scores of defoliation (first to fifth score); N—the total number of trees; K – maximum score on the scale. The forest ecosystems are considered to be damaged at C > 30% [121] Percentage of defoliation is determined by sight with guidance, where the habitus of crowns with different rates of defoliation for each tree species is given.

Dendro-chronological analysis for ecosystem health assessment. By the dendrochronological analysis, the impact of external factors (including contaminants) on the radial growth of stems may be determined. Depending on the change of these variables, the characteristic pattern of the tree ring series was formed. The pattern includes: successively alternating narrow and wide rings of lighter or darker wood; changes in the density of the tree rings; change in the ratio between early and late wood, changes in the chemical, cytological and histological characteristics of tree rings, etc. The year with changes in growth or with special annual ring, is named *different special year*. The different special years are very important in cross-dating and in identifying the age of trees, as well as the time of stressed events by climatic factors, pollution, disease, pests, etc. The samples are taken with Presler's driller on 1–1.5 m height of the stem and placed in special templates. They are measured by LINTAB™. Through the statistical processing of obtained series of values, the influence of considered stressors or bio assessment can be performed [121]. The main indicator for dendro-chronogical, especially dendrochemical analysis, is the growth index (It). It is the ratio between measured and calculated value for tree ring (Wt/Gt) by the best reflected to the course of tree stem growth trend ($R^2 > 0.45$). Thus, the influence of age on the growth is eliminated and the environmental information in the rings is enhanced. The analysis of content of chemical elements in annual rings and its dynamics can provide valuable information about changes in the environmental toxicity. There is information for over 70 chemical elements that can be absorbed from the soil by root system, also through the bark or caught by the leaves and moved to the xylem. Many authors have found that the vertical transport of nutrients thousand times exceeds the horizontal one, that is, radial migration of elements in annual rings is minimal, because they form the insoluble

complexes [1, 3, 111 and others]. Therefore, the content of chemical elements in the wood gives a general picture of the environmental factors influenced on tree species for their lifetime. The chemical memory of annual rings also can be used to estimate the changes in the environmental toxicity. Recently, the set of dendrochronological indicators have been developed for the forest state assessments: number of eustress periods; their duration, frequency and depth; eustress years (unfavourable climatic type of years), reactive tree functional types and eustress-climatic predictive patterns [122–124]. The authors perceive eustress as a repeating state of restricted radial growth rate of tree stems within a period of one or multiple years and caused by unfavourable environmental factors. This state encompasses the numerous other reactions of tree species. The level of radial stem growth (or tree ring width) is the main parameter that the developed holistic approach operates with, as well as the growth index, which is the main indicator for the statistical determination of low growth threshold (categorized as eustress). The study of the forest ecosystem state is based on the assessment of eustress depth (A) – $A = \frac{1}{s}\sum_{i=1}^{s}(1 - It_i)$, duration (D) – the number of adjacent eustress years, and frequency (F) – the number of stress years for a period of 100 years, and the creation of eustress nomenclature by five-graded scales. The performance evaluation of eustress in particular localities allows the expression of reactive functional type of tree species. For example, functional type F4D5A4 means that in particular locality the typical for trees of that species are frequent, very long and deep eustresses and that "forest behaviour" puts the existence of the forest under some risk. For the analysis of eustress based on the periods with limited growth, SP-PAM 2.0 software has been developed [125]. Thus, these analyses can be applied for the forest ecosystem state assessment.

Nowadays, *the kits* are widely used for short-, long-term and risk ecotoxicological assessment, as well as for ecosystem health assessment and for quality monitoring of water and wastewater, because they are rapid, sensitive and cost-effective way [126–128]; determining the impact of bio-toxins produced by blue-green algae [129–131], chemicals for mutagenicity [132], chemicals and wastes released in aquatic, terrestrial environments and sediments [127]. Different model organisms are used like algae, aquatic invertebrates, bacteria and plants.

4. Conclusion

New synthetic chemicals are recorded each year and the legislation in countries requires the immediate conduction of the both – toxicological and ecotoxicological testing. The scale of the potential ecological impacts on the environment and biota requires fast and accurate assessments of toxicological effects. The practical importance of ecotoxicology for the existence and functioning of the MBS is constantly growing. The toxicity may be different for different species in the ecosystem and for the same species in different ecosystems. Furthermore, toxicants do not only directly affect the biological system being evaluated, but may have an indirect negative effect on it, altering both abiotic and biotic parameters in the ecosystem. The various populations of the same species under different environmental conditions will respond differently to a given concentration of toxicant. In ecotoxicology practice, the number of species is used as

test objects, and the results are extrapolated to all groups of organisms in the ecosystem. The variation in size, physiology, evolution, ontogeny and geographical distribution are some of the important parameters that usually do not fit exactly. However, some of the basic tests have demonstrated its great importance in the understanding of contaminants effects on the environment. The series of variables must be considered for the realistic assessment of environmental toxicity and MBS state. The reported sublethal effects often refer to changes in the structure of MBS that can lead to their degradation. A greater variation in the responses of individuals, populations and ecosystems observed in nature are compared with these reported under laboratory conditions, due to the mutual influence. This fact requires the more intensive usage of multi-testing systems—micro- and mesocosms and new developments. The analysis of situation and problems of ecotoxicological testing makes it possible to outline the directions in which to focus future efforts. They are related to the search of sensitive species for acute and risk testing, developing of new biomarkers and kits, especially for the study of terrestrial toxicity, formation of model systems (micro- and mesocosms) by key members of the ecosystem trophic network for multi-species testing and modelling the toxic effects at MBS level, which is especially true for the terrestrial ecotoxicology.

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References

[1] Lyubenova M, Kalchev R. Ecotoxicology – Small Practicum (second amended edition). An-Di Press, Sofia; 2009. 305 p. (in Bulgarian)

[2] Newman MC, Clements WH. editors. Ecotoxicology: A Comprehensive Treatment. CRC Press, Taylor & Francis Group; 2008. 852 p

[3] Lyubenova M. Plant Ecology. "M. Drinov" Academic Press, Sofia; 2004. 574 p. (in Bulgarian)

[4] Lyubenova M. Short Course of Functional Biocoenology. Marketing Press, Sofia; 2009. 319 p. (in Bulgarian)

[5] Lyubenova M. About ecological fundaments of new generation global models. Comptes rendus de l'Academie Bulgare des Sciences. Biologie, Ecologie, 2011;64:1589–1601 (available at http://www.proceedings.bas.bg/)

[6] Whitefield AM, Simpson MJ, Simpson AJ, Zeeb BA, Rutter A. Earthworm metabolomic responses after exposure to aged PCB contaminated soils. Ecotoxicology. 2012;21:1947–1956. doi: 10.1007/s10646-012-0928-x

[7] Parolini M, Binelli A. Sub-lethal effects induced by a mixture of three non-steroidal anti-inflammatory drugs (NSAIDs) on the freshwater bivalve Dreissena polymorpha. Ecotoxicology. 2012;21:379–392. doi: 10.1007/s10646-011-0799-6

[8] Zhang C, Li F, Xiang J. Acute effects of cadmium and copper on survival, oxygen consumption, ammonia-N excretion, and metal accumulation in juvenile Exopalaemon carinicauda. Ecotoxicology and Environmental Safety. 2014;104:209–214. doi: 10.1016/j.ecoenv.2014.01.008

[9] Oliveira IB, Beiras R, Thomas KV, Suter MJ.-F, Barroso CM. Acute toxicity of tralopyril, capsaicin and triphenylborane pyridine to marine invertebrates. Ecotoxicology. 2014;23:1336–1344. doi: 10.1007/s10646-014-1276-9

[10] Yao Y, Lu Z, Min H, Gao H, Zhu F. The effect of tetrahydrofuran on the enzymatic activity and microbial community in activated sludge from a sequencing batch reactor. Ecotoxicology. 2012;21:56–65. doi: 10.1007/s10646-011-0765-3

[11] McMurry ST, Jones LE, Smith PN, Cobb GP, Anderson TA, Lovern MB, Cox S, Pan X. Accumulation and effects of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) exposure in the green anole (Anolis carolinensis). Ecotoxicology. 2012;21:304–314. doi: 10.1007/s10646-011-0791-1

[12] Davies R, Zou E. Polybrominated diphenyl ethers disrupt molting in neonatal Daphnia magna. Ecotoxicology. 2012;21:1371–1380. doi: 10.1007/s10646-012-0891-6

[13] Méndez-Fernández L, Martínez-Madrid M, Rodriguez P. Toxicity and critical body residues of Cd, Cu and Cr in the aquatic oligochaete Tubifex tubifex (Müller) based on lethal and sublethal effects. Ecotoxicology. 2013;22:1445–1460. doi: 10.1007/s10646-013-1131-4

[14] Sun KF, Xu XR, Duan SS, Wang YS, Cheng H, Zhang ZW, Zhou GJ, Hong YG. Ecotoxicity of two organophosphate pesticides chlorpyrifos and dichlorvos on non-targeting cyanobacteria Microcystis wesenbergii. Ecotoxicology. 2015;24:1498–1507. doi: 10.1007/s10646-015-1458-0

[15] Bezirci G, Akkas SB, Rinke K, Yildirim F, Kalaylioglu Z, Severcan F, Beklioglu M. Impacts of salinity and fish-exuded kairomone on the survival and macromolecular profile of Daphnia pulex. Ecotoxicology. 2012;21:601–614. doi: 10.1007/s10646-011-0820-0

[16] Breckels RD, Neff BD. Pollution-induced behavioural effects in the brown bullhead (Ameiurus nebulosus). Ecotoxicology. 2010;19:1337–1346. doi: 10.1007/s10646-010-0520-1

[17] Fonseca AP, Marques EJ, Torres JB, Silva LM, Siqueira HÁ. Lethal and sublethal effects of lufenuron on sugarcane borer Diatraea flavipennella and its parasitoid Cotesia flavipes. Ecotoxicology. 2015;24:1869–1879. doi: 10.1007/s10646-015-1523-8

[18] Carneiro M, Gutiérrez-Praena D, Osório H, Vasconcelos V, Carvalho AP, Campos A. Proteomic analysis of anatoxin-a acute toxicity in zebrafish reveals gender specific responses and additional mechanisms of cell stress. Ecotoxicology and Environmental Safety. 2015;120:93–101. doi: 10.1016/j.ecoenv.2015.05.031

[19] Li AJ, Leung PTY, Bao VWW, Yi AXL, Leung KMY. Temperature-dependent toxicities of four common chemical pollutants to the marine medaka fish, copepod and rotifer. Ecotoxicology. 2014;23:1564–1573. doi: 10.1007/s10646-014-1297-4

[20] Penttinen S, Malk V, Väisänen A, Penttinen OP. Using the critical body residue approach to determine the acute toxicity of cadmium at varying levels of water hardness and dissolved organic carbon concentrations. Ecotoxicology and Environmental Safety. 2011;74:1151–1155. doi: 10.1016/j.ecoenv.2011.03.017

[21] Kim J, Park J, Kim PG, Lee C, Choi K, Choi K. Implication of global environmental changes on chemical toxicity-effect of water temperature, pH, and ultraviolet B irradiation on acute toxicity of several pharmaceuticals in Daphnia magna. Ecotoxicology. 2010;19:662–669. doi: 10.1007/s10646-009-0440-0

[22] Sprague JB. The ABC's of pollutant bioassay using fish. In: Cairns Jr J, Dickson KL, editors. Biological Methods for the Assessment of Water Quality. American Society for Testing and Materials (ASTM), Philadelphia PA. STP 528; 1973. p. 6–30

[23] Pérez S, Rial D, Beiras R. Acute toxicity of selected organic pollutants to saltwater (mysid Siriella armata) and freshwater (cladoceran Daphnia magna) ecotoxicological models. Ecotoxicology. 2015;24:1229–1238. doi: 10.1007/s10646-015-1489-6

[24] Jang S, Ji K. Effect of chronic exposure to two components of Tritan copolyester on Daphnia magna, Moina macrocopa, and Oryzias latipes, and potential mechanisms of endocrine disruption using H295R cells. Ecotoxicology. 2015;24:1906–1914. doi: 10.1007/s10646-015-1526-5

[25] Cuhra M, Traavik T, Dando M, Primicerio R, Holderbaum D, Bøhn T. Glyphosate-Residues in Roundup-Ready Soybean Impair Daphnia magna Life-Cycle. Journal of Agricultural Chemistry and Environment. 2015;4:24–36. doi: 10.4236/jacen.2015.41003

[26] Bettinetti R, Croce V, Noè F, Ponti B, Quadroni S, Galassi S. Ecotoxicity of pp'DDE to Daphnia magna. Ecotoxicology. 2013;22:1255–1263. doi: 10.1007/s10646-013-1113-6

[27] Ventura SP, Gonçalves AM, Sintra T, Pereira JL, Gonçalves F, Coutinho JA. Designing ionic liquids: the chemical structure role in the toxicity. Ecotoxicology. 2013;22:1–12. doi: 10.1007/s10646-012-0997-x

[28] Jesus FT, Oliveira R, Silva A, Catarino AL, Soares AM, Nogueira AJ, Domingues I. Lethal and sub lethal effects of the biocide chlorhexidine on aquatic organisms. Ecotoxicology. 2013;22:1348–1358. doi: 10.1007/s10646-013-1121-6

[29] Thyrring J, Juhl BK, Holmstrup M, Blicher ME, Sejr MK. Does acute lead (Pb) contamination influence membrane fatty acid composition and freeze tolerance in intertidal blue mussels in arctic Greenland? Ecotoxicology. 2015;24:2036–2042. doi: 10.1007/s10646-015-1539-0

[30] Fang Q, Shi X, Zhang L, Wang Q, Wang X, Guo Y, Zhou B. Effect of titanium dioxide nanoparticles on the bioavailability, metabolism, and toxicity of pentachlorophenol in zebrafish larvae. Journal of Hazardous Materials. 2015;283:897–904. doi: 10.1016/j.jhazmat.2014.10.039

[31] Domingues I, Oliveira R, Soares AM, Amorim MJ. Effects of ivermectin on Danio rerio: a multiple endpoint approach: behaviour, weight and subcellular markers. Ecotoxicology. 2016;25:491–499. doi: 10.1007/s10646-015-1607-5

[32] Nunes B, Braga MR, Campos JC, Gomes R, Ramos AS, Antunes SC, Correia AT. Ecotoxicological effect of zinc pyrithione in the freshwater fish Gambusia holbrooki. Ecotoxicology. 2015;24:1896–1905. doi: 10.1007/s10646-015-1525-6

[33] Wu Q, Li M, Gao X, Giesy JP, Cui Y, Yang L, Kong Z. Genotoxicity of crude extracts of cyanobacteria from Taihu Lake on carp (Cyprinus carpio). Ecotoxicology. 2011;20:1010–1017. doi: 10.1007/s10646-011-0670-9

[34] Saucedo-Vence K, Dublán-García O, López-Martínez LX, Morachis-Valdes G, Galar-Martínez M, Islas-Flores H, Gómez-Oliván LM. Short and long-term exposure to diclofenac alter oxidative stress status in common carp Cyprinus carpio. Ecotoxicology. 2015;24:527–539. doi: 10.1007/s10646-014-1401-9

[35] Atli G, Canli M. Metals (Ag+, Cd2+, Cr6+) affect ATPase activity in the gill, kidney, and muscle of freshwater fish Oreochromis niloticus following acute and chronic exposures. Environmental Toxicology. 2013;28:707–717 doi: 10.1002/tox.20766

[36] Chen WY, Lin CJ, Ju YR, Tsai JW, Liao CM. Coupled dynamics of energy budget and population growth of tilapia in response to pulsed waterborne copper. Ecotoxicology. 2012;21:2264–2275. doi: 10.1007/s10646-012-0983-3

[37] Arena M, Sgolastra F. A meta-analysis comparing the sensitivity of bees to pesticides. Ecotoxicology. 2014;23:324–334. doi: 10.1007/s10646-014-1190-1

[38] Alayrangues J, Hotier L, Massou I, Bertrand Y, Armengaud C. Prolonged effects of in-hive monoterpenoids on the honey bee Apis mellifera. Ecotoxicology. 2016;25:856–862. doi: 10.1007/s10646-016-1642-x

[39] Li D, Liu Z, Cui Y, Li W, Fang H, Li M, Kong Z. Toxicity of cyanobacterial bloom extracts from Taihu Lake on mouse, Mus musculus. Ecotoxicology. 2011;20:1018–1025. doi: 10.1007/s10646-011-0693-2

[40] Rattner BA, Horak KE, Lazarus RS, Eisenreich KM, Meteyer CU, Volker SF, Campton CM, Eisemann JD, Johnston JJ. Assessment of toxicity and potential risk of the anticoagulant rodenticide diphacinone using Eastern screech-owls (Megascops asio). Ecotoxicology. 2012;21:832–846. doi: 10.1007/s10646-011-0844-5

[41] Cardone A. Imidacloprid induces morphological and molecular damages on testis of lizard (Podarcis sicula). Ecotoxicology. 2015;24:94–105. doi: 10.1007/s10646-014-1361-0

[42] Cesar R, Natal-da-Luz T, Silva F, Bidone E, Castilhos Z, Polivanov H, Sousa JP. Ecotoxicological assessment of a dredged sediment using bioassays with three species of soil invertebrates. Ecotoxicology. 2015;24:414–423. doi: 10.1007/s10646-014-1390-8

[43] de García SO, García-Encina PA, Irusta-Mata R. Dose-response behavior of the bacterium Vibrio fischeri exposed to pharmaceuticals and personal care products. Ecotoxicology. 2016;25:141–162. doi: 10.1007/s10646-015-1576-8

[44] Manzo S, Miglietta ML, Rametta G, Buono S, Di Francia G. Toxic effects of ZnO nanoparticles towards marine algae. Science of the Total Environment. 2013;445–446:371–376. doi: 10.1016/j.scitotenv.2012.12.051

[45] Teodorović I, Knežević V, Tunić T, Cučak M, Lečić JN, Leovac A, Tumbas II. Myriophyllum aquaticum versus Lemna minor: sensitivity and recovery potential after exposure to atrazine. Environmental Toxicology and Chemistry. 2012;31:417–426. doi: 10.1002/etc.748

[46] Vannini C, Domingo G, Marsoni M, De Mattia F, Labra M, Castiglioni S, Bracale M. Effects of a complex mixture of therapeutic drugs on unicellular algae Pseudokirchneriella subcapitata. Aquatic Toxicology. 2011;101:459–465. doi: 10.1016/j.aquatox.2010.10.011

[47] Qian H, Li J, Pan X, Sun Z, Ye C, Jin G, Fu Z. Effects of streptomycin on growth of algae Chlorella vulgaris and Microcystis aeruginosa. Environmental Toxicology. 2012;27:229–237. doi: 10.1002/tox.20636

[48] Jiang HS, Li M, Chang FY, Li W, Yin LY. Physiological analysis of silver nanoparticles and AgNO3 toxicity to Spirodela polyrhiza. Environmental Toxicology and Chemistry. 2012;31:1880–1886. doi: 10.1002/etc.1899

[49] Oukarroum A, Bras S, Perreault F, Popovic R. Inhibitory effects of silver nanoparticles in two green algae, Chlorella vulgaris and Dunaliella tertiolecta. Ecotoxicology and Environmental Safety. 2012;78:80–85. doi: 10.1016/j.ecoenv.2011.11.012

[50] Sánchez-Fortún S, López-Rodas V, Navarro M, Marvá F, D'ors A, Rouco M, Haigh-Florez D, Costas E. Toxicity and adaptation of Dictyosphaerium chlorelloides to extreme chromium contamination. Environmental Toxicology and Chemistry/SETAC. 2009;28:1901–1905. doi: 10.1897/08-489.1

[51] DeLorenzo ME, Scott GI, Ross PE. Toxicity of pesticides to aquatic microorganisms: a review. Environmental Toxicology and Chemistry. 2001;20:84–98

[52] Polonini HC, Brandão HM, Raposo NR, Mouton L, Yéprémian C, Couté A, Brayner R. Ecotoxicological studies of micro- and nanosized barium titanate on aquatic photosynthetic microorganisms. Aquatic Toxicology. 2014;154:58–70. doi: 10.1016/j.aquatox.2014.05.005

[53] Hartmann NB, Von der Kammer F, Hofmann T, Baalousha M, Ottofuelling S, Baun A. Algal testing of titanium dioxide nanoparticles--testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology. 2010;269:190–197. doi: 10.1016/j.tox.2009.08.008

[54] Byzitter J, Lukowiak K, Karnik V, Dalesman S. Acute combined exposure to heavy metals (Zn, Cd) blocks memory formation in a freshwater snail. Ecotoxicology. 2012;21:860–868. doi: 10.1007/s10646-011-0847-2

[55] Mohammed EH, Wang G, Jiang J. The effects of nickel on the reproductive ability of three different marine copepods. Ecotoxicology. 2010;19:911–916. doi: 10.1007/s10646-010-0471-6

[56] Fogel MN, Schneider MI, Desneux N, González B, Ronco AE. Impact of the neonicotinoid acetamiprid on immature stages of the predator Eriopis connexa (Coleoptera: Coccinellidae). Ecotoxicology. 2013;22:1063–1071. doi: 10.1007/s10646-013-1094-5

[57] Freitas EC, Rocha O. Acute and chronic effects of sodium and potassium on the tropical freshwater cladoceran Pseudosida ramosa. Ecotoxicology. 2011;20:88–96. doi: 10.1007/s10646-010-0559-z

[58] Liu YR, Zheng YM, He JZ. Toxicity of profenofos to the springtail, Folsomia candida, and ammonia-oxidizers in two agricultural soils. Ecotoxicology. 2012;21:1126–34. doi: 10.1007/s10646-012-0867-6

[59] Svartz GV, Herkovits J, Pérez-Coll CS. Sublethal effects of atrazine on embryolarval development of Rhinella arenarum (Anura: Bufonidae). Ecotoxicology. 2012;21:1251–1259. doi: 10.1007/s10646-012-0880-9

[60] Almond KM, Trombetta LD. The effects of copper pyrithione, an antifouling agent, on developing zebrafish embryos. Ecotoxicology. 2016;25:389–398. doi: 10.1007/s10646-015-1597-3

[61] Liu TX, Zhang Y. Side effects of two reduced-risk insecticides, indoxacarb and spinosad, ontwo species of Trichogramma (Hymenoptera: Trichogrammatidae) on cabbage. Ecotoxicology. 2012;21:2254–2263. doi: 10.1007/s10646-012-0981-5

[62] Navis S, Waterkeyn A, Voet T, De Meester L, Brendonck L. Pesticide exposure impacts not only hatching of dormant eggs, but also hatchling survival and performance in the water flea Daphnia magna. Ecotoxicology. 2013;22:803–814. doi: 10.1007/s10646-013-1080-y

[63] Harbeitner RC, Hahn ME, Timme-Laragy AR. Differential sensitivity to pro-oxidant exposure in two populations of killifish (Fundulus heteroclitus). Ecotoxicology. 2013;22:387–401. doi: 10.1007/s10646-012-1033-x

[64] Clark BW, Di Giulio RT. Fundulus heteroclitus adapted to PAHs are cross-resistant to multiple insecticides. Ecotoxicology. 2012;21:465–474. doi: 10.1007/s10646-011-0807-x

[65] Collard HR, Ji K, Lee S, Liu X, Kang S, Kho Y, Ahn B, Ryu J, Lee J, Choi K. Toxicity and endocrine disruption in zebrafish (Danio rerio) and two freshwater invertebrates (Daphnia magna and Moina macrocopa) after chronic exposure to mefenamic acid. Ecotoxicology and Environmental Safety. 2013;94:80–86. doi: 10.1016/j.ecoenv.2013.04.027

[66] Soares J, Castro LF, Reis-Henriques MA, Monteiro NM, Santos MM. Zebrafish (Danio rerio) life-cycle exposure to chronic low doses of ethinylestradiol modulates p53 gene transcription within the gonads, but not NER pathways. Ecotoxicology. 2012;21:1513–1522. doi: 10.1007/s10646-012-0905-4

[67] Howe PL, Reichelt-Brushett AJ, Clark MW. Effects of Cd, Co, Cu, Ni and Zn on asexual reproduction and early development of the tropical sea anemone Aiptasia pulchella. Ecotoxicology. 2014;23:1593–1606. doi: 10.1007/s10646-014-1299-2

[68] Ouellet JD, Dubé MG, Niyogi S. Influence of elevated alkalinity and natural organic matter (NOM) on tissue-specific metal accumulation and reproductive performance in fathead minnows during chronic, multi-trophic exposures to a metal mine effluent. Ecotoxicology and Environmental Safety. 2013;95:104–112. doi: 10.1016/j.ecoenv.2013.05.027

[69] le Mer C, Roy RL, Pellerin J, Couillard CM, Maltais D. Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (Gasterosteus aculeatus). Ecotoxicology and Environmental Safety. 2013;89:174–181. doi: 10.1016/j.ecoenv.2012.11.027

[70] Kovrižnych JA, Sotníková R, Zeljenková D, Rollerová E, Szabová E. Long-term (30 days) toxicity of NiO nanoparticles for adult zebrafish Danio rerio. Interdisciplinary Toxicology. 2014;7: 23–26. doi: 10.2478/intox-2014-0004

[71] Benamú MA, Schneider MI, González A, Sánchez NE. Short and long-term effects of three neurotoxic insecticides on biological and behavioural attributes of the orb-web spider Alpaida veniliae (Araneae, Araneidae): implications for IPM programs. Ecotoxicology. 2013;22:1155–1164. doi: 10.1007/s10646-013-1102-9

[72] Bommarito T, Sparling DW, Halbrook RS. Toxicity of coal-tar pavement sealants and ultraviolet radiation to Ambystoma Maculatum. Ecotoxicology. 2010;19:1147–1156. doi: 10.1007/s10646-010-0498-8

[73] Mustafa SA, Al-Subiai SN, Davies SJ, Jha AN. Hypoxia-induced oxidative DNA damage links with higher level biological effects including specific growth rate in

common carp, Cyprinus carpio L. Ecotoxicology. 2011;20:1455–1466. doi: 10.1007/s10646-011-0702-5

[74] Al Kaddissi S, Legeay A, Elia AC, Gonzalez P, Camilleri V, Gilbin R, Simon O. Effects of uranium on crayfish Procambarus clarkii mitochondria and antioxidants responses after chronic exposure: what have we learned? Ecotoxicology and Environmental Safety. 2012;78:218–224. doi: 10.1016/j.ecoenv.2011.11.026

[75] Velisek J, Sudova E, Machova J, Svobodova Z. Effects of sub-chronic exposure to terbutryn in common carp (Cyprinus carpio L.). Ecotoxicology and Environmental Safety. 2010;73:384–390. doi: 10.1016/j.ecoenv.2009.10.005

[76] Azizullah A, Richter P, Jamil M, Häder DP. Chronic toxicity of a laundry detergent to the freshwater flagellate Euglena gracilis. Ecotoxicology. 2012;21:1957–1964. doi: 10.1007/s10646-012-0930-3

[77] Li ZH, Li P, Shi ZC. Responses of the hepatic glutathione antioxidant defense system and related gene expression in juvenile common carp after chronic treatment with tributyltin. Ecotoxicology. 2015;24:700–705. doi: 10.1007/s10646-014-1416-2

[78] Chen WY, Liao CM. Interpreting copper bioaccumulation dynamics in tilapia using systems-level explorations of pulsed acute/chronic exposures. Ecotoxicology. 2014;23:1124–1136. doi: 10.1007/s10646-014-1255-1

[79] Koelmans AA, Hubert E, Koopman HW, Portielje R, Crum SJH, Modeling sediment porewater profiles of carbendazim in indoor freshwater microcosms. Environmental Toxicologu and Chemistry. 2000;19:793–800

[80] Biney C, Amuzu AT, Calamari D, Kaba N, Mbome IL. Review of heavy metals, in report of review of pollution in the African aquatic FAO. Rapport sur les Peches (FAO). 1992;471:7–45

[81] Dekker T, Greve GD, Ter Laak TL, Boivin ME, Veuger B, Gortzak G, Dumfries S, Lücker SMG, Kraak MHS, Admiraal W, van der Geest HG. Development and application of a sediment toxicity test using the benthic cladoceran Chydorus sphaericus. Environmental Pollution. 2006;140:231–238. doi: 10.1016/j.envpol.2005.07.015

[82] Borgmann U, Norwood WP, Reynoldson TB, Rosa F. Identifying cause in sediment assessments: bioavailability and the sediment quality triad. Canadian Journal of Fisheries and Aquatic Sciences. 2001;58:950–960

[83] Piva F, Ciaprini F, Onorati F, Benedetti M, Fattorini D, Ausili A, Regoli F. Assessing sediment hazard through a weight of evidence approach with bioindicator organisms: a practical model to elaborate data from sediment chemistry, bioavailability, biomarkers and ecotoxicological bioassays. Chemosphere. 2011;83:475–485. doi: 10.1016/j.chemosphere.2010.12.064

[84] Gomiero A, Dagnino A, Nasci C, Viarengo A. The use of protozoa in ecotoxicology: application of multiple endpoint tests of the ciliate E. crassus for the evaluation of

sediment quality in coastal marine ecosystems. The Science of Total Environment. 2013;442:534–544. doi: 10.1016/j.scitotenv.2012.10.023

[85] Maranho LA, Garrido-Pérez MC, Baena-Nogueras RM, Lara-Martín PA, Antón-Martín R, DelValls TA, Martín-Díaz ML. Are WWTPs effluents responsible for acute toxicity? Seasonal variations of sediment quality at the Bay of Cádiz (SW, Spain). Ecotoxicology. 2015;24:368–380. doi: 10.1007/s10646-014-1385-5

[86] Brinkmann M, Hudjetz S, Kammann U, Hennig M, Kuckelkorn J, Chinoraks M, Cofalla C, Wiseman S, Giesy JP, Schäffer A, Hecker M, Wölz J, Schüttrumpf H, Hollert H. How flood events affect rainbow trout: evidence of a biomarker cascade in rainbow trout after exposure to PAH contaminated sediment suspensions. Aquatic Toxicology. 2013;128–129:13–24. doi: 10.1016/j.aquatox.2012.11.010

[87] Vega-López A, Ayala-López G, Posadas-Espadas BP, Olivares-Rubio HF, Dzul-Caamal R. Relations of oxidative stress in freshwater phytoplankton with heavy metals and polycyclic aromatic hydrocarbons.Comparative Biochemistry and Physiology Part A Molecular and Integrative Physiology. 2013;165:498–507. doi: 10.1016/j.cbpa.2013.01.026

[88] Beyger L, Orrego R, Guchardi J, Holdway D. The acute and chronic effects of endosulfan pulse-exposure on Jordanella floridae (Florida flagfish) over one complete life-cycle. Ecotoxicology and Environmental Safety. 2012;76:71–78. doi: 10.1016/j.ecoenv.2011.09.015

[89] Jordaan MS, Reinecke SA, Reinecke AJ. Acute and sublethal effects of sequential exposure to the pesticide azinphos-methyl on juvenile earthworms (Eisenia andrei). Ecotoxicology. 2012;21:649–661. doi: 10.1007/s10646-011-0821-z

[90] Vighi M, Villa S. Ecotoxicology: the challenges for the 21st century. Toxics 2013;1:18–35. doi: 10.3390/toxics1010018

[91] Speck-Planche A, Kleandrova VV, Luan F, Cordeiro MNDS. Predicting multiple ecotoxicological profiles in agrochemical fungicides: a multi-species chemoinformatic approach. Ecotoxicology and Environmental Safety. 2012;80:308–313. doi: 10.1016/j.ecoenv.2012.03.018 PMID:22521812

[92] Minguez L, Di Poi C, Farcy E, Ballandonne C, Benchouala A, Bojic C, Cossu-Leguille C, Costil K, Serpentini A, Lebel JM, Halm-Lemeille MP. Comparison of the sensitivity of seven marine and freshwater bioassays as regards antidepressant toxicity assessment. Ecotoxicology. 2014;23:1744–1754. doi: 10.1007/s10646-014-1339-y

[93] Bastos AC, Prodana M, Abrantes N, Keizer JJ, Soares AM, Loureiro S. Potential risk of biochar-amended soil to aquatic systems: an evaluation based on aquatic bioassays. Ecotoxicology. 2014;23:1784–1793. doi: 10.1007/s10646-014-1344-1

[94] Gagnaire B, Boyer P, Bonzom JM, Lecomte-Pradines C, Simon O, Gilbin R. Transfer modelling and toxicity evaluation of the effluent from an installation of cleansing and

uranium recovery using a battery of bioassays. Ecotoxicology. 2011;20:187–201. doi: 10.1007/s10646-010-0570-4

[95] Lyubenova M, Dineva S, Georgieva N, Georgieva T, Karadjova I, Parvanova P.. Ecotoxicology assessment model of cultural plant-soil complex treated with waste water. Biotechnology and Biotechnological Equipment. 2012;26:2883–2893(available at http://www.tandfonline.com/loi/tbeq20#.V5of1Lh942w)

[96] Lyubenova M, Dineva S, Karadjova I. Ecotoxicology assessment of waste water emitted from Radomir metal industries (Bulgaria). Ecologia Balkanica. 2012;4: 51–60(available at http://eb.bio.uni-plovdiv.bg/en/)

[97] Clément L, Hurel C, Marmier N. Toxicity of TiO2 nanoparticles to cladocerans, algae, rotifers and plants: effects of size and crystalline structure. Chemosphere. 2013;90:1083–1090 doi: 10.1016/j.chemosphere.2012.09.013

[98] Basiuk EV, Ochoa-Olmos OE, De la Mora-Estrada LF. Ecotoxicological effects of carbon nanomaterials on algae, fungi and plants. Journal of Nanoscience and Nanotechnology. 2011;11:3016–3038

[99] Saison C, Perreault F, Daigle JC, Fortin C, Claverie J, Morin M, Popovic R. Effect of core-shell copper oxide nanoparticles on cell culture morphology and photosynthesis (photosystem II energy distribution) in the green alga, Chlamydomonas reinhardtii. Aquatic Toxicology. 2010;96:109–114. doi: 10.1016/j.aquatox.2009.10.002

[100] Velki M, Hackenberger BK. Species-specific differences in biomarker responses in two ecologically different earthworms exposed to the insecticide dimethoate. Comparative Biochemistry and Physiology. Toxicology and Pharmacology: CPB. 2012;156:104–112. doi: 10.1016/j.cbpc.2012.05.001

[101] Almeida JR, Oliveira C, Gravato C, Guilhermino L. Linking behavioural alterations with biomarkers responses in the European seabass Dicentrarchus labrax L. exposed to the organophosphate pesticide fenitrothion. Ecotoxicology. 2010;19:1369–1381. doi: 10.1007/s10646-010-0523-y

[102] Lin D, Li Y, Zhou Q, Xu Y, Wang D. Effect of triclosan on reproduction, DNA damage and heat shock protein gene expression of the earthworm Eisenia fetida. Ecotoxicology. 2014;23:1826–1832. doi: 10.1007/s10646-014-1320-9

[103] Chankova S, Mitrovska Z, Miteva D, Oleskina YP, Yurina NP. Heat shock protein HSP70B as a marker for genotype resistance to environmental stress in Chlorella species from contrasting habitats. Gene. 2013;516:184–189. doi: 10.1016/j.gene.2012.11.052

[104] de Moraes FD, de Figueiredo JSL, Rossi PA, Venturini FP, Moraes G. Acute toxicity and sublethal effects of phenol on hematological parameters of channel catfish Ictalurus punctatus and pacu Piaractus mesopotamicus. Ecotoxicology and Environmental Contamination. 2015;10:31–36 doi: 10.5132/eec.2015.01.05

[105] Denoël M, D'Hooghe B, Ficetola GF, Brasseur C, De Pauw E, Thomé JP, Kestemont P. Using sets of behavioral biomarkers to assess short-term effects of pesticide: a study

case with endosulfan on frog tadpoles. Ecotoxicology. 2012;21:1240–1250. doi: 10.1007/s10646-012-0878-3

[106] Sacchi A, Mouneyrac C, Bolognesi C, Sciutto A, Roggieri P, Fusi M, Beone GM, Capri E. Biomonitoring study of an estuarine coastal ecosystem, the Sacca di Goro lagoon, using Ruditapes philippinarum (Mollusca: Bivalvia). Environmental Pollution. 2013;177:82–89. doi: 10.1016/j.envpol.2013.01.042

[107] Kolkmeier MA, Brooks BW. Sublethal silver and NaCl toxicity in Daphnia magna: a comparative study of standardized chronic endpoints and progeny phototaxis. Ecotoxicology. 2013;22:693–706. doi: 10.1007/s10646-013-1061-1

[108] Feito R, Valcárcel Y, Catalá M. Biomarker assessment of toxicity with miniaturised bioassays: diclofenac as a case study. Ecotoxicology. 2012;21:289–296. doi: 10.1007/s10646-011-0790-2

[109] Lyubenova M, Roumenina E, Dimitrov V, Ivanov E. Research on ecosystems of the biosphere reserve "Chuprene" with plant ecological methods and spatial modeling – B: coll. Scientific International Conference in Memory of Prof. Dr. Dimitar Yaranov, Varna, 2002;1:260–269. (in Bulgarian)

[110] Roumenina E, Lyubenova M, Dimitrov V. Ecological risk assessment of spruce vegetation in the biosphere reserve "Chuprene" through spatial modeling in GIS. In: Coll. International Conference – "75 years Forest institute', Sofia, 1–5 October 2003;1: 61–64

[111] Lyubenova M, Valtcheva T, Pachedjieva K. Assessment of the risk for the beech vegetation in the biosphere reserve "Chouprene" connected with climatic, orographic and soil factors. Annuire de l'Universite de Sofia "St. Kliment Ohridski", 2005;96:27–35

[112] Lyubenova M, Nedkov R, Ivanova I, Chikalanov A, Georgieva N, Zaharinova M, Dimitrova M, Ivanova E, Yanchev V, Radeva K, Stankova N, Tsoneva R. 2. Study on ecological dynamics of forest vegetation in the region East Rhodope on the base of satellites and terrestrial data. Ecological Engineering and Environmental Protection. 2011;1:45–50(available at http://ecoleng.org/EEEP.html)

[113] Lyubenova M, Nedkov R, Georgieva N, Dineva S. Space models of oak vegetation in protected zone, Bulgaria. Indian Journal of Applied Research. 2014;4:23–30(available at http://www.worldwidejournals.com/indian-journal-of-applied-research-(IJAR)/)

[114] Lyubenova M, Assenova A, Bardarova K. Condition of communities of juniper in Bulgaria (II). In: Coll. Scientific Reports International Conference "50 Years University of Forestry', Sofia, 1–2 April 2003, Department of Ecology and Environmental Protection. 2003;122–126. (in Bulgarian)

[115] Lyubenova M. Basic approaches to ecosystem diagnostics. Ecological Engineering and Environmental Protection. 2004;3:16–23

[116] Lyubenova M, Ivanova V, Bratanova-Doncheva S. Ecosystem Assessment of Chestnut Forests in Belasitsa. An–Di Publisher, Sofia. 2011; 112 p. (in Bulgarian)

[117] Georgieva N, Lyubenova M. Assessment of ecological status of xerothermic oak ecosystems in SCI "Zapadna Stara planina i Predbalkan. Bulgarian Journal of Agricultural Science, 2014;20(Supplement 1):105–109(available at http://www.agrojournal.org/)

[118] Lyubenova M, Peteva S. Management and services of forest ecosystem: benefits, payment and values. Journal of Balkan Ecology. 2016;19(1):35–49

[119] Lyubenova M, Dimitrova V. Chemical elements in mulch and litter-fall of beech ecosystems and their total turnover. Ecologia Balkanica. 2011;3:51–57

[120] Lyubenova M, Bratanova–Doncheva S, Dimitrova V, Grozeva M. Main characteristics of biological turn-over in Castanea sativa Mill: communities from Belasitza mountain, Bulgaria. Acta Horticulture. 2010;866:275–284

[121] Mirchev S, Lyubenova M, Shikalanov A, Simeonova N. Dendrochronology. Short Course. Pensoft, Sofia-Moscow. 2000; 198 p. (in Bulgarian)

[122] Lyubenova M. Plant Functional Types of Trees (PFTs) According to the Features of Low Growth (Eustress) Periods. "PablishSaySetEco" Publishing house, Sofia. 2012; 276 p. (in Bulgarian)

[123] Lyubenova M. Holistic analysis on eustress of Quercus rubra L. and Quercus robur L. Journal of Balkan Ecology. 2014;17:391–401

[124] Lyubenova M, Chikalanov A, Lyubenova V. Holistic ecological assessment of dendrochronological series as source of information for tree eustress (example with beech). International Journal of Environmental Monitoring and Protection. 2014;1:24–34 (available at http://www.openscienceonline.com/journal/ijemp)

[125] Lyubenova M, Chikalanov A, Lyubenova V, Vatov S. SPPAM 2.0 – Scientific description and use. Journal of Environmental Science, Computer Science and Engineering & Technology, Sec.B. 2015; 4(1):37–51 (available at http://www.jecet.org/)

[126] Polyseed. InterLab supply [Internet]. 2016. Available from: http://www.polyseed.com/tds/POLYTOX%20TDS%20updated%2003.05.15.pdf [Accessed: 2016-06-19]

[127] MicroBoitests Inc. Toxkit Microbiotests [Internet]. 2016. Available from: http://www.microbiotests.be/ [Accessed: 2016-06-19]

[128] Bio Hidrica. Biotecnologías del Agua Ltda. [Internet]. 2016. Available from: http://www.biohidrica.cl/english/biohidrica_productsenvironment_water.htm [Accessed: 2016-06-19]

[129] Lai EPC. Bioassay kits for ecotoxicological testing of wastewaters with nanoparticles. Biochemistry and Analytical Biochemistry. 2013;2:e139. doi: 10.4172/2161-1009.1000e139

[130] Abraxis [Internet]. 2016. Available from: http://www.abraxiskits.com/products/algal-toxins/ [Accessed: 2016-06-19]

[131] EnviroLogixTM [Internet]. 2016. Available from: http://www.envirologix.com/wp-content/uploads/2015/05/ET022-Microcystin-101215.pdf [Accessed: 2016-06-19]

[132] National Toxicology Program. U. S. Department of Health and Human Services [Internet]. 2016. Available from: http://ntp.niehs.nih.gov/testing/types/genetic/invitro/sa/index.html [Accessed: 2016-06-19]

Chapter 8

A Review of Cyanogenic Glycosides in Edible Plants

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Additional information is available at the end of the chapter

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Abstract

Cyanogenic glycosides are natural plant toxins that are present in several plants, most of which are consumed by humans. Cyanide is formed following the hydrolysis of cyanogenic glycosides that occur during crushing of the edible plant material either during consumption or during processing of the food crop. Exposure to cyanide from unintentional or intentional consumption of cyanogenic glycosides may lead to acute intoxications, characterized by growth retardation and neurological symptoms resulting from tissue damage in the central nervous system (CNS). Processing methods can detoxify cyanogenic glycosides and reduce the risk of cyanide poisoning. The efficiency of cyanide removal, however, depends on the processing technique employed and the extent of processing. Processing operations such as fermentation, boiling/cooking, and drying, applied to process food-containing cyanogenic glycosides have been reported to reduce cyanide content to acceptably safe levels. The present review discusses the level of cyanogenic glycosides in specific plant foods, health implications of consuming cyanogenic plants and effect of various processing method on cyanogenic glycosides with updated information gathered from the published reports on cyanogenic glycosides.

Keywords: cyanogenic glycosides, cyanide, toxins, processing, cyanide poisoning, food safety

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1. Introduction

Food plants contain many substances that can pose potential risks to consumers, and one of these types of substances is cyanogenic glycosides. Cyanogenic glycosides are chemical compounds contained in foods that release hydrogen cyanide when chewed or digested. The act of chewing or digestion leads to hydrolysis of the substances, causing cyanide to be released [1]. Cyanogenic glycosides are common in certain families such as the Fabaceae, Rosaceae, Leguminosae, Linaceae, and Compositae, and identification of their constituents is a useful tool for informative taxonomic markers [2]. There are approximately 25 known cyanogenic glycosides and these are generally found in the edible parts of plants, such as apples, apricots, cherries, peaches, plums, quinces, particularly in the seed of such fruits. The chemicals are also found in almonds, stone fruit, pome fruit, cassava, bamboo shoots, linseed/flaxseed, lima beans, coco yam, chick peas, cashews, and kirsch [3, 4]. Other food products that may contain cyanogenic glycosides include some food ingredients with flavoring properties such as ground almonds powder or paste, marzipan, stone fruit, and alcoholic drinks made from stone fruits. These foods therefore represent potential sources of hydrogen cyanide [5].

The toxicity of cyanogenic glycosides and their derivatives is dependent on the release of hydrogen cyanide. Toxicity may result in acute cyanide poisoning and has also been implicated in the etiology of several chronic diseases [6, 7]. Cyanide toxicity can occur in animal including humans at doses between 0.5 and 3.5 mg HCN per kilogram body weight. Symptoms of cyanide toxicity in humans have been reported to include vomiting, stomach ache, diarrhea, convulsion, and in severe cases death [5]. Children are particularly at risk because of their smaller body size [1].

The toxicity of cyanogenic glycosides is associated with their ability to be hydrolyzed either spontaneously or in the presence of enzyme to produce cyanide as end products of their hydrolysis. Thus, toxic levels of cyanogenic glycosides are estimated in terms of the quantity of free cyanide generated following hydrolysis. This makes it difficult to estimate total cyanogenic glycosides in diet. Hence, levels of amygdalin (the most common cyanogenic glycosides in fruits) reported in literature are inconsistent [8]. Although the level of cyanide up to 10ppm was reported by World Health Organization (WHO) to be safe for cassava flour [9]. Lack of quantitative toxicological test and epidemiological information makes it difficult to establish safe level of cyanogenic glycosides intake in many foods.

Cyanogenic glycosides are a group of nitrile-containing plant secondary compounds that yields cyanide (cyanogenesis) following their enzymatic breakdown. Cyanogenic glycosides occur in at least 2000 plant species, of which a number of species are used as food. They are amino-acid-derived constituents of plants produced as secondary metabolites [5]. Despite great deal of structural diversity in cyanogenic glycosides, almost all of them are believed to be derived from only six different amino acids L-valine, L-isoleucine, L-leucine, L-phenylalanine, or L-tyrosine, and cyclopentenyl-glycine (a nonprotein amino acid) [2, 10]. Cyanogenic glycosides play pivotal roles in organization of chemical defence system in plants and in plant-insect interactions [10].

Cyanogenic glycoside is not toxic on its own. However, when cell structures of plant are disrupted, cyanogenic glycoside will be brought together with the corresponding β-glucosidase enzyme. Hydrolysis of cyanogenic glycosides usually occurs when cyanogenic plants are chewed by herbivores or when the plants are disintegrated during processes, such as grinding, pounding or in the presence of water for example during soaking or fermentation [11]. Hydrolysis is accomplished by the β-glucosidase, producing sugars and a cyanohydrin that spontaneously decomposes to HCN and a ketone or aldehyde [11, 12]. Different kinds of cyanogenic glycosides may be found in different cyanogenic food plants, for example, taxiphyllin in bamboo shoots, linamarin in cassava [5]. Several commercial crop plants, such as sorghum (*Sorghum bicolor*), apple, apricot, almond kernels cassava (*Manihot esculenta*), and barley (*Hordeum vulgare*), are cyanogenic and accumulate significant amounts of cyanogenic glycosides [2, 13, 14]. Generally, the level of cyanogenic glycosides produced is dependent upon the age and the variety of the plant, as well as environmental factors [2].

2. Cyanogenic plant foods

2.1. Cassava

High levels of cyanogenic glycosides have been reported to be present in many nutritious dense plants, thus limiting the use of some of the plants as food. For instance, cassava (*Manihot esculenta*) is one of such plant with high amount of cyanogenic glycosides. It is a crop of economic importance in Africa and a staple food in most African communities [15]. Cassava is also a valued crop in South America and Southeast Asia. The high carbohydrate content of the crop makes it a good source of calorie for feeding billions of people in the world [16]. According to Food and Agriculture organization, cassava is the third most important source of calories in the tropics, after rice and corn [1, 9]. Cassava leaves have higher protein content, contain vitamin C and vitamin A and provide some dietary fiber [17]. Much of the protein in the leaves is made up of linamarase, the enzyme that detoxifies the cyanogenic glycosides in cassava [18]. However, each parts of cassava plants (leaves, stem, root) contains high levels of cyanogenic glycosides; linamarin, lotaustralin, and amygdalin [14, 19], with linamarin been the most predominant cyanogen [19].

The cyanide level of cassava varies from about 75 to 350 ppm, but can be up to l000 ppm or more depending on the variety, plant age, soil condition, fertilizer application, weather, and other factors [20]. Studies have shown that the levels of cyanogenic glycosides in cassava roots are generally lower than that in the leaves and stems [21, 22]. Cassava roots have been reported to contain cyanide content of 10–500 mg/kg of dry matter [23] and the leaves were reported to contain 53–1300 cyanide equivalents/kg of dry matter [24].

Cassava root is processed into various forms before consumption. The root can be boiled and eaten as whole root, or processed into flour for the production of "thick gruel" commonly consumed in Africa. It can also be converted into chips for the production of flour, tapioca, and other foods. Cassava leaves are used in some African countries to produce soup or sauce, following processes such as scrubbing, fermentation, pounding, and boiling. Apart from

human consumption, cassava roots and peels are also consumed by animal [1]. The major cyanogenic glycoside in cassava is linamarin, while small amount of lotaustralin (methyl linamarin) and amygdalin are also present, as well as an enzyme linamarase. Linamarin is rapidly hydrolyzed by linamarase to glucose, acetone cyanohydrin, and hydrogen cyanide (**Figure 1**).

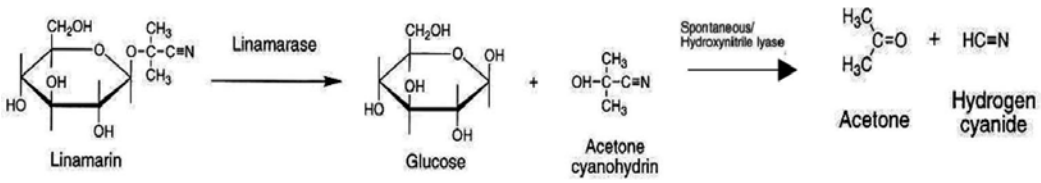

Figure 1. Hydrolysis of linamarin to produce hydrogen cyanide.

Under neutral conditions, acetone cyanohydrin decomposes to acetone and hydrogen cyanide. Although processing methods can reduce linamarin and cyanide in food, improperly processed cassava products would contain some amount of residual linamarin and hydrogen cyanide. This would results in the potential toxicity of the cassava products. Indeed, cases of cyanide toxicity from the consumption of inadequately processed cassava products have been reported [18, 25]. Recent studies have reported the presence of cyanogenic glycoside, amygdalin in cassava [14]. Amygdalin content of 8.84–48.33 mg/g has been reported to be present in cassava roots, depending on variety [14]. Bitter cassava varieties are more drought resistant and thus more readily available and cheaper but contain high level of cyanogenic glucosides. However, owing to food shortage in times of drought, less time is available for the additional processing required for cassava products. This leads to cassava products with high cyanogenic level with the potential of affecting consumers' health. Increasing the production of cassava breed with low cyanogen content [10] will improve healthy livelihoods of cassava producers, processors and consumers.

2.2. Cocoyam

Edible cocoyam is a nutrient dense tuber crop that belongs to Araceae family. Two major species of this family are Taro (*Colocasia esculenta L*) and Tannia (*Xanthosoma Sagittifolium* L). These species are commonly grown in the tropical region of Africa and are generally referred to as cocoyam [26]. Cocoyams are valued for its corms, cormels, and leaves. It can be converted into flour and used to make mashed meal or porridge; it can be consumed baked or boiled. It is a rich source of calorie for millions of people in the tropical and subtropical regions due to its high carbohydrate content [26, 27]. In addition to carbohydrate, cocoyam also contains other sources of nutrients such as protein, vitamins, carotenoids and minerals [9]. Apart from the nutrient composition of cocoyam tuber, the crops have been reported to contain low levels (2.10–17.13 mg/100g) of cyanogenic glycosides [28, 29]. In another study, cocoyam tuber was reported to contain 7.4 mg/100g equivalent cyanide [27].

2.3. Bamboo shoot

Fresh immature bamboo shoots are consumed as vegetable in some Asian countries. Bamboo shoot contains appreciable quantities of vitamin C, carbohydrates, and protein [30]. Besides its nutritive value, bamboo shoots also contain lethal concentration of cyanogenic glycosides. The cyanogenic glycoside present in bamboo shoot is taxiphyllin, which is decomposed quickly in boiling water. Cyanide content of bamboo shoot ranged from 1000 to 8000 mg/kg hydrogen cyanide [5, 31]. Although cyanide content of bamboo shoot is much higher than that of cassava root, the cyanide content in bamboo shoots decreases substantially following harvesting and processing [32].

2.4. Sorghum (*Sorghum bicolor*)

Dhurrin is the major cyanogenic glucoside in sorghum, representing 30% of the dry weight of shoot tips of seedlings, in addition to amygdalin [14]. In young sorghum leaves, dhurrin is localized in vacuoles of plants and the enzymes responsible for its hydrolysis to hydrogen cyanide is located in cytoplasm. In intact leaf, tissues are free from cyanide due to compartmental separation of the enzyme and the substrate. Concentration of dhurin decreases as plant ages, immature sorghum leaves contains high concentration of dhurrin [33]. The cyanide content of sorghum increases rapidly during early growth stage, after which it declines with plant age [34]. The reports by [35, 36] confirmed the cyanogenic glucoside content in the tip of young seedlings of *Sorghum bicolor* that reaches 6% of the dry weight. Sorghum grains have been reported to contain 122.31 mg/g of amygdalin [14].

2.5. Fruits and fruits kernels

The most common cyanogenic glycoside in fruits and fruit kernel is amygdalin. Amygdalin contents of the fruit seeds vary significantly from seed to seed [13, 14, 37].

2.5.1. Apple (Malus domestica)

It is the most cherished fruit among the Rosaceae family. Apples can be consumed raw or processed into alcoholic (cider) or nonalcoholic beverage (apple juice) or apple sauce. Although apples are rich sources of vitamins and other nutrients, apple seeds contain high levels of cyanogenic glycosides. Amygdalin content of apple seeds ranged from 1 to 4 mg/g while that of apple juice was reported to be between 0.001 and 0.08 mg/ml [13, 37].

2.5.2. Apricot fruits (Prunus armeniaca)

They are cultivated in the Middle Asia, Africa, America, and Europe. The fruits are consumed raw or used in dry form for confectioneries while the kernels are usually processed before consumption. Apricot kernels are of two varieties; bitter and sweet. Bitter apricot kernels contain high amount of the cyanogenic glycosides, amygdalin, which can cause cyanide toxicity problems at high dose and thus, unsafe for consumption. Sweet apricot kernels as well as apricot flesh are safe for human consumption because of their low level of cyanogens [13, 38]. The concentration of hydrogen cyanide in Apricot kernels varies widely (49–4000 mg/kg),

depending on whether skin on or off varieties is surveyed. Cyanide level of raw or improperly processed apricot kernels can cause serious acute problems that could lead to death [4, 3]. Studies have shown that apricot kernel contain cyanide (CN) content of 1450 mg/kg, approximately 0.5 mg CN/kernel [39]. This value is similar to the toxic dose of cyanide (0.5 mg/kg body weight) reported by [5].

3. Health implications of cyanogenic glycosides

The toxicity of cyanogenic glycosides and their derivatives is dependent on the release of hydrogen cyanide. Toxicity may result in acute cyanide poisoning and has also been implicated in the etiology of several chronic diseases [7]. Dietary exposure to elevated levels of some cyanogenic glycosides in food has the potential to cause acute cyanide poisoning or a debilitating irreversible neurological condition in the long term.

3.1. Konzo

High and sustained cyanogens intake at sublethal concentrations from cassava or cassava flour in combination with a low intake of sulfur amino acids has been reported to cause Konzo in women and children [32]. Konzo is an upper motor neuron disease characterized by irreversible but nonprogressive symmetric spastic paraparesis that has an abrupt onset. It mostly affects children and women of childbearing age [40–42].

3.2. Tropical ataxic neuropathy (TAN)

It is another health problem associated with continuous consumption of improperly processed cassava products. TAN is used to describe several neurological syndromes attributed to toxiconutritional causes. TAN has occurred mainly in Africa, particularly Nigeria [43] and is common among people of 40 years and above [32]. Dietary exposure to cyanide from the monotonous consumption of inadequately processed cassava products over years is responsible for the cause of the disease. Symptoms of TAN include sore tongue, optical atrophy, neuro sensory deafness, and sensory gait ataxia [43].

3.3. Goiter and cretinism

They are common diseases in developing countries due to low intake of iodine (<100 μg/day). The disease is particularly common in Africa because of their over dependent on cassava as a staple food. Continuous exposure to dietary cyanide from insufficiently processed cassava products aggravate the disease [44] by the interferences of thiocyanate (the end products of cyanide detoxification in human system) with dietary iodine, thus leading to iodine deficiency. According to reference [44], populations with very low iodine intake and high thiocyanate levels from consumption of cassava, showed severe endemic goiter, which decreases with iodine supplementation. Study has shown that consumption of cyanogenic glycosides even at a very low concentration can also cause iodine deficiency leading to goiter [45].

3.4. Growth retardation

It is a common health problem especially among children in developing countries. Exposure to cyanogenic glycosides has been a contributing factor to this health problem. Growth retardation is particularly a serious problem in populations consuming foods with inadequate proteins especially diets that are low in sulfur containing amino acids (methionine and cysteine). This is because detoxification of cyanide in human body requires sulfur donors from sulfur-containing amino acids [46]. Thus, dietary exposure to cyanide is a contributing factor to growth retardation [47].

3.5. Cyanide poisoning

Cyanide toxicity occurs when cytochrome oxidase a_3 inhibits the terminal enzyme in the respiratory chain and halts electron transport and oxidative phosphorylation (which is essential to the synthesis of adenosine triphosphate (ATP) and the continuation of cellular respiration) [48, 49]. Cyanide poisoning occurs as a result of consumption of bitter cassava, almond kernels, or apricot kernels and their products without proper processing. Cases of cyanide poisoning after consumption of drink produced from the blends of apricot kernels and orange juice have been reported [50]. Clinical symptoms of cyanide toxicity are vomiting, nausea, dizziness, stomach pains, weakness, headache, diarrhea, and occasionally death [4, 51–54].

4. Effect of processing on cyanogenic glycosides

Processing methods, such as peeling, drying, grinding, soaking, boiling or cooking, soaking and fermentation have been reported by several studies to cause significant reduction in the cyanogenic glycosides of processed foods. These processes have been applied to food crops such as roots, tubers, cereals, and leaves, to cause significant reduction in the cyanogen contents of the crops. Food-processing methods generally disintegrate cyanogens contents of plants, and this leads to the production of cyanide. Since cyanide is volatile, further processing techniques, such as roasting and drying, will volatilize the remaining cyanide to low level.

4.1. Effect of soaking on cyanogenic glycosides

Soaking of cassava root has been reported to decrease its total cyanide content by 13 52% after 24 h, 73–75% after 48 h and 90% after 72 h [55]. Ref. [56] reported that endogenous β-glucosidase activity causes a significant degradation of amygdalin in ground apricot kernels soaked at 20°C.

4.2. Effect of fermentation on cyanogenic glycosides

Fermentation of cassava pulp or dough for 4–5 days has been reported to decrease its total cyanide by 52–63% [55]. Soaking and fermentation of bitter apricot kernels decreased cyanogen levels by about 70% [57]. The cyanide content of cocoyam flour produced from fermented

cocoyam was reported to reduce by 98.6% [27]. Prasad and Dhanya [58] reported a 84.6% reduction in the cyanide content of fermented sorghum leaves.

4.3. Effect of storage on cyanogenic glycosides

When foods produced from cyanogenic plants are stored at room temperature (35 ± 2°C), cyanide contents of the foods volatilize due to its low temperature (26°C). Ref [59] reported a decrease of 50–64% in the cyanide content of a cassava product (gari) stored for 4 weeks at room temperature.

4.4. Effect of cooking on cyanogenic glycosides

Cyanogenic glycosides are generally water soluble. During cooking, significant amount of cyanogens are leached into cooking water. Several studies have reported increased reduction of cyanide in cooked products. Steaming of a cassava product (akyeke) was reported to result in a 74–80% reduction in total cyanide levels. "Garification", a process whereby fermented and dried cassava mash is simultaneously cooked and dried in a shallow wok, resulted in a 90–93% reduction in total cyanide content. Optimal cooking conditions for the reduction of cyanide levels in bamboo shoots (98–102°C for 148–180 min) resulted in a 97% reduction in cyanide [31]. Generally, traditional African processes typically decrease the cyanide content of cassava by 97–>99%. Also when the cooking method chosen is heating under dry, heat or at low moisture contents, the intake of the cyanogen is limited to only small amounts.

4.5. Effect of drying on cyanogenic glycosides

The efficiency of cyanide removal during drying is dependent on moisture content of the roots, rate of moisture loss (which relates to drying conditions), and the extent of tissue disruption of the plant tissue [60]. Extending the period of drying with higher moisture levels would enhanced linamarin breakdown, thus explaining the fact that fast drying rates result in lower detoxification, while slower rates result in higher cyanogen removal. Famurewa and Emuekele [61] reported that the higher moisture contents of the cassava root the greater the loss in cyanide content during drying. cutting of cassava tubers into small chips might create easy access for contact between the enzymes and cyanogenic glycosides resulting in higher hydrolysis. Reduction in cyanide content could also be as a result of variety, maturity and product sizes [62].

5. Cyanide detoxification in human

Cyanide is detoxified in the body by the enzyme rhodanase with the help of sulfur-containing amino acids to thiocyanate, which is excreted in the urine [63]. However, the detoxication mechanism of cyanide in the human body can only cope with low level of cyanide generated from consumption of small amount of cyanogenic plants.

6. Conclusion

Cyanogenic glycosides are abundant in edible plants. Consumption of improperly processed cyanogenic plants can lead to chronic and acute health problems. Understanding the appropriate processing methods for specific cyanogenic plants will help in reducing the problem of unintentional cyanide toxicity. Similarly, to prevent adverse effects of cyanogenic glycosides in food plants, consumers should prepare foods properly before consumption. It is recommended that cyanogenic plants should be cut into smaller pieces and cook thoroughly to release toxic hydrogen cyanide before consumption in order to reduce the level of the toxin. Also, seeds of fruits to be processed into juice should be removed before crushing to avoid cyanide poisoning.

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References

[1] Kwok J. Cyanide poisoning and cassava. Food safety focus. (19th issue February, 2008). Incident focus. (2008). http://www.cfs.gov.hk/english/multimedia

[2] Vetter J. Plant cyanogenic glycosides. Toxicon, 2000; 38: 11-36.

[3] Codex Committee on Contaminants in Foods. Discussion paper on cyanogenic glycosides. CX/CF 09/3/11. FAO/WHO, Rome. Codex Committee on Contaminants in Foods. (2008) Discussion paper on cyanogenic glycosides. CX/CF 09/3/11. 2008. Rome: FAO/WHO.

[4] Haque MR, Bradbury JH. Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food Chemistry, 2002; 77: 107-114.

[5] WHO: World Health Organization. Cyanogenic glycosides. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additive Series 30. Geneva. 1993. Available from: http://www.inchem.org/documents/jecfa/jecmono/v30je18.htm

[6] Cressey P, Saunders D. Determination of presence of cyanogenic residues in apple juices in Australia and New Zealand. June 2012. Institute of Environmental Science &

Research Limited contracted by the New Zealand Ministry for Primary Industries. 2012. Unpublished. Report FW12032.

[7] FAO/WHO. WHO Food Additive Series: 65. Safety evaluation of certain food additives and contaminants. Prepared by the 74th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2012. World Health Organization, Geneva.

[8] WHO: World Health Organization. Hydrogen cyanide and cyanides: Human health aspects. 2004. Concise International Chemical Assessment Document 61, Geneva.

[9] Food and Agricultural Organisation (FAO). Roots, tubers, plantain and bananas in human nutrition. Food and Agricultural Organisation of the United Nation. 1991; 7(1): 11-21.

[10] Ganjewala D, Kuma S, Asha DS, Ambika K. Advances in cyanogenic glycosides biosynthesis and analyses in plants: a review. Acta Biol Szeged, 2010; 54: 1–14.

[11] CFS (Centre for Food Safety). Natural toxins in food plants. Risk assessment studies. Food and Environmental Hygiene Department, the Government of the Hong Kong Special Administrative Region. Report No. 27. 2007.

[12] Gruhnert C, Biehl B, Selmar D. Compartmentation of cyanogenic glucosides and their degrading enzymes. Planta, 1994; 195: 36-42.

[13] Bolarinwa IF, Orfila C, Morgan MRA. Determination of amygdalin in apple seeds, fresh apples and processed apple juices. Food Chem. 2015; 170: 437-442.

[14] Bolarinwa IF, Olaniyan SA, Olatunde SJ, Ayandokun FT, Olaifa IA. Effect of processing on amygdalin and cyanide contents of some Nigerian Foods. J Chem Pharm Res, 2016; 8(2): 106-113.

[15] Nhassico D, Muquingue H, Cliff J, Cumbana A, Bradbury JH. Rising African cassava production, diseases due to high cyanide intake and control measures. J Sci Food Agric, 2008; 88: 2043-2049.

[16] Nweke F. New challenges in the cassava transformation in Nigeria and Ghana. Environment and Production Technology Division, International Food Policy Research Institute, USA. www.ifpri.org.2004; p. 1-118.

[17] South Pacific Trade Commission. Exporting Fruit and Vegetables to New Zealand, A Guide for Pacific Island Producers. Auckland, New Zealand. 2000.

[18] Bokanga M, Ekanayake IJ, Dixon AGO, Porto MCM. Genotype-environment interactions for cyanogenic potential in cassava. Acta Hort, 1994; 375: 131-139.

[19] Lykkesfeldt J, Moller BL. Cyanogenic glycosides in cassava, Manihot esculenta Crantz. Acta Chem Scanda, 1994; 48: 178-180.

[20] Gomez G, Valdivieso M. The effect of variety and plant age on cyanide content, chemical composition and quality of cassava roots. Nutr Rep Int 1983; 27(4): 857-865.

[21] Riis L, Bellotti AC, Bonierbale M, O'Brien GM. Cyanogenic potential in cassava and its influence on a generalist insect herbivore (Cyrtomenus bergi Hemiptera: Cydnidae). J Econ Entomol., 2003; 96: 1905-1914.

[22] Nambisan B. Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety. Food Chem Toxicol, 2011; 49: 690-693.

[23] Siritunga D, Sayre RT. Generation of cyanogen-free transgenic cassava. Planta, 2003; 217: 367-373.

[24] Wobeto C, Correa AD, De Abreu CMP, Dos Santos CD, Pereira HV. Anti-nutrients in the cassava (Manihot esculenta Crantz) leaf powder at three ages of the plant. Cien Technol Aliment, 2007; 27: 108-112.

[25] Akintonwa A, Tunwase OL. Fatal cyanide poisoning from cassava based-meal. Hum Exp Toxicol, 1992; 11: 47-49.

[26] Nail H. Understanding the production of the major tropical/ subtropical root crops: cassava, potatoes, yams and cocoyams. Technical Paper, China, 2010. p. 17-35.

[27] Igbadul BD, Amoye J, Twadue I. Effect of fermentation on the proximate composition, antinutritional factors and functional properties of cocoyam (*Colocasia esculenta*) flour. Afr J Food Sci Technol, 2014; 5(3): 67-74.

[28] Abdulrashid M, Agwunobi LN. Taro cocoyam (*Colocasia esculenta*) meal as feed ingredient in poultry. PJN, 2009; 8: 666-673,

[29] Olajide R, Akinsoyinu AO, Babayemi O J, Omojola A B, Abu AO, Afolabi K D. Effect of processing on energy value, nutrient and anti-nutrient components of wild cocoyam (*Colocasia esculenta* (L.) Scchot) corm. PJN, 2011; 10: 29-34.

[30] Bhargava A, Kumbhare V, Srivastava A, Sahai A. Bamboo parts and seeds for additional source nutrition. J Food Sci Technol, 1996; 32: 145-146.

[31] Ferreira VLP, Yotsuyanagi K, Carvalho CRL. Elimination of cyanogenic compounds from bamboo shoots *Dendrocalamus giganteus* Munro. Trop Sci, 1995; 35: 342-346.

[32] FSANZ. Final assessment report proposal P257. Advice on the preparation of cassava and bamboo shoots. 2004. Report Number 2-04. Canberra.

[33] Poulton JE. Cyanogenesis in plants–review. Plant Physiol, 1990; 94: 401-405.

[34] Busk PK, Møller BL. Dhurrin synthesis in sorghum is regulated at the transcriptional level and induced by nitrogen fertilization in older plant. Plant Physiol, 2002; 129: 1222-1231.

[35] Akazawa T, Miljanich P, Conn EE. Studies on cyanogenic glycosides of *Sorghum vulgare*. Plant Physiol., 1960; 35: 535-538.

[36] Halkier BA, Møller BL. Biosynthesis of the cyanogenic glucoside dhurrin in seedlings of *Sorghum bicolor* (L.) Moench and partial purification of the enzyme system involved. Plant Physiol, 1989; 90: 1552-1559.

[37] Bolarinwa IF, Orfila C, Morgan MRA. Amygdalin content of seeds kernels and food products commercially-available in the UK. Food Chem, 2014; 152: 1333-139.

[38] Canadian Food Inspection Agency. Cyanides in bitter apricot kernels. 2009. Available from: http://www.hc-sc.gc.ca/fn-an/pubs/securit/2009-apricots-abricots/index-eng.php

[39] Holzbecher MD, Moss MA, Ellenberger HA. The cyanide content of laetrile preparations, apricot, peach and apple seeds. Clin Toxicol, 1984; *22*: 341-347.

[40] Ministry of Health, Mozambique Mantakassa. An epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area Mozambique. Nutritional factors and hydrocyanic acid content of cassava products. Bull World Health Org, 1984; 62: 485-492.

[41] Tylleskar T, Banae M, Bikangi N, Cooke RD, Poulter NH, Rosling H. Cassava cyanogens and konzo, an upper motorneuron disease found in Africa. Lancet, 1992; 339: 208-211.

[42] Ernesto M, Cardoso AP, Nicala D, Mirione E, Massaza F, Cliff J, Hague MR, Bradbury JH. Persistent konzo and cyanogens toxicity from cassava in northern Mozambique. Acta Trop, 2002; 82: 357-362.

[43] Oluwole OSA, Onabolu AO, Link H, Rosling H. Persistence of tropical ataxic neuropathy in a Nigerian community. J Neurol Neurosurg Psychiatry, 2000; 69: 96-101.

[44] Rosling H. Cassava Toxicity and Food Security. Ed. Rosling. Tryck Kontakt, Uppsala, Sweden. 1987. p. 3-40.

[45] Odo CE, Enechi OC, Oburu CS. Concentrations of anti-nutritional factors in raw edible cocoyam (*Colocasia esculenta*) leaves. J Pharm Res, 2014; 8(1): 38-40.

[46] Rosling H. Measuring effects in humans of dietary cyanide exposure from cassava. Acta Hortic, 1994; 375: 271-283.

[47] Banea-Mayambu JP, Tylleskar, T, Tylleskar K, Gebre-Medhin M, Rosling H. Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo (formerly Zaire). Ann Trop Pediatr, 2000; 20: 34-40.

[48] Beasley DMG, Glass WI. Cyanide poisoning: pathophysiology and treatment recommendations. Occup Med, 1998; 48: 427-431.

[49] Hamel J. A review of acute cyanide poisoning with a treatment update. Crit Care Nurse, 2011; 31: 72-82.

[50] Atkinson K. Apricot kernels carry risk of cyanide poisoning. 2006. Available from: http://www.nzherald.co.nz/organisation/story.cfm?o_id=324&objectid=10379786.

[51] Lash EE, Shawa RE. Multiple cases of cyanide poisoning by apricot kernels in children from Gaza. Pediatrics, 1981; 68: 5-7.

[52] Mlingi N, Poulter NH, Rosling, H. An outbreak of acute intoxications from consumption of insufficiently processed cassava in Tanzania. Nutr Res, 1992; 12: 677-687.

[53] Akyildiz BN, Kurtoğlu S, Kondolot M, Tunç A. Cyanide poisoning caused by ingestion of apricot seeds. Ann Trop Paediatr, 2010; 30: 39-43.

[54] Sanchez-Verlaan P, Geeraerts T, Buys S, Ruy-Poulenc B, Cabot, C, Fourcade O, Megarbane B, Genestral M. An unusual cause of severe lactic acidosis: cyanide poisoning after bitter almond ingestion. Intens Care Med, 2011; 37: 168-169.

[55] Kemdirimi OCC, Chukwu OA, Anchinewhu SC. Effect of traditional processing of cassava on the cyanide content of gari and cassava flour. Plant Foods Hum Nutr, 1995; 48: 335-339.

[56] Tuncel G, Nout MJR, Brimer L. The effects of grinding, soaking and cooking on the degradation of amygdalin of bitter apricot seeds. Food Chem, 1995; 53: 447-451.

[57] Tuncel G, Nout MJR, Brimer L. Degradation of cyanogenic glycosides of bitter apricot seeds (Prunus armeniaca) by endogeneous and added enzymes as affected by heat treatments and particle size. Food Chem, 1995; 63: 65-69.

[58] Prasad S, Dhanya MS. Determination and detoxification of cyanide content in sorghum for ethanol production using *Saccharomyces Cerevisiae* strain. J Metabol Syst Biol, 2011; 2(1): 10-14.

[59] Onabolu AO, Oluwole OSA, Rosling H, Bokanga M. Processing factors affecting the level of residual cyanohydrins in gari. J Sci Food Agric, 2002; 82: 966-969

[60] Tivana LD, Bvochora J, Mutukumira AN, Owens JD, Zvauya R. Heap fermentation of cassava (Manihot Esculenta Crantz) in Nampula province, Mozambique. Proceedings of the 13th ISTRC Symposium, 2007; 445 – 450.

[61] Famurewa JAV, Emuekele PO. Cyanide reduction pattern of cassava (*Manihot esculenta*) as affected by variety and air velocity using fluidized bed dryer. Afr J Food Sci Technol, 2014; 5(3): 75-80.

[62] Cock JH. Cassava: New Potential for a Neglected Crop. West View Press, Boulder, CO, USA. 1985.

[63] Banea MJP, Nahimana G, Mandombi C, Bradbury JH, Denton IC, Kuwa, N. Control of konzo in DRC using the wetting method on cassava flour. Food Chem Toxicol, 2012; 50: 1517-1523.

Chapter 9

Toxic Effects as a Result of Herbal Medicine Intake

Nudrat Fatima and Naira Nayeem

Additional information is available at the end of the chapter

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Abstract

Concurrent use of herbs with therapeutic drugs increases the potential of herb-drug interactions. The clinical importance of herb-drug interactions is associated with the particular herb, drug, and patient profile. Herbs are potentially potent as they affect body functions. The use herbal medicine and supplements can be risky as they are not subject to review by the FDA. In this chapter, we make an attempt to discuss the possible reasons for toxic effects, types of toxicities, some reported cases of toxicities involving the use of herbal medicine alone, and some herb-drug interactions. In addition to this, possible ways to reduce toxic effects of herbal medicines have also been discussed.

Keywords: herbal medicine, toxicity, reported cases, herb-drug interaction

1. Introduction

Herbal medicines are advertised to be free from side effects, which is a myth. A large number of people still rely on herbal medicines, and some people take herbal medicines along with routine allopathic medicines especially in cases of diabetes, hypertension, thyroid disease, etc., where the patient is on long-term or lifelong treatment. Many commercial websites are available on Internet, which insist that herbal medicines have no side effects. In underdeveloped and developing countries, there are no specific laws for herbal practitioners and companies marketing herbal products. People are attracted by such companies and start using herbal medicines pertaining to be free from side effects. In this chapter, we will discuss about possible toxicities of some herbal medicines and their remedies.

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2. Possible reasons for toxic effects of herbal medicines

2.1. Self-treatment

Herbal medicines are easily available in market and can be purchased without prescription. These products are advertised on media as a miracle treatment without any side effects to attract people that are fed up with side effects or lost hope for being cured. The patients who like to play safe game are attracted in a manner that they are allowed to continue their regular medicines along with herbal treatment. Even persons caring about their health start herbal treatment to remain healthy proving the proverb "Prevention is better than cure." As a result, a large number of people are attracted towards herbal medicines and they start self-treatment [1].

2.2. Unqualified practitioners

In a large part of the world, unqualified practitioners are prescribing alternative therapies to patients of various diseases, apart from some countries where laws and regulations for herbal practitioners exist and implemented. Medical practitioners are provided vast knowledge about human body, drugs, mechanism of action, pharmacology, case studies, and then allowed to practice. Nowadays, Alternative Medicine Degree Course is available in certain universities with highly qualified and experienced faculty, which is a good source of herbal practitioners, but still 50% of herbal practitioners in the world are unqualified who acquire this profession after their forefathers such as a son of a farmer becomes a farmer, or after reading some books about herbal medicine, conducting 6-month online course about herbal medicine, etc., and start practicing. In underdeveloped countries, people are attracted towards these quacks due to economic reasons and start taking herbal medicine. These unqualified practitioners themselves are not aware of toxic effects of herbal medicines, and if the patient complains, they cannot rectify their mistake.

2.3. Sub-standard product

There are many sub-standard herbal products available in the market. The reason is that these products are not tested accordingly for quality before marketing. Some contain less amount of active ingredient and some do not contain active ingredient at all as a result of incorrect identification of plant by the collector, using adulterant instead of original plant or due to improper storage of plant material, and it loses its efficacy. Sometimes the herbal products contain material not defined on label such as non-herb material, minerals, heavy metals, and addition of particular pharmaceutical product. Occasionally they may contain toxins and pesticides, which is much more dangerous and one of the major reasons of toxic effects after herbal medicine intake [2].

2.4. Improper intake

Allopathic medicines are marketed after extensive testing and trials, and their dose is fixed according to age and weight of the patient. All possible adverse effects are listed in leaflet. But

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no such procedures are followed in case of herbal medicines. Some of herbal medicines are considered as dietary supplements, and proper dose is not mentioned. No measure cup or spoon provided with the medicine as in case of allopathic syrups. Usually, same dose is applied for persons of different age and weight. Companies selling these products misguide people and claim their product totally free from adverse effects. Even there is no period mentioned, some people continue for months or years, which in long term can be harmful for human health [3].

3. Types of toxicities

3.1. Nephrotoxicity

Drug or toxin causing kidney damage upon exposure to a certain level and the kidneys are unable to pass excess urine, and waste product is the condition termed as nephrotoxicity. In this condition, there is an elevation in blood electrolytes such as potassium and magnesium. This condition starts temporarily but, if not detected earlier, could be severe. Nephrotoxicity can be detected by two simple tests of blood urea nitrogen (BUN) and creatinine levels in blood together termed as kidney function tests. Normal range values of BUN and creatinine are 10–25 mg/dl and 0.7–1.4 mg/dl, respectively. These values may increase due to following factors:

a. Dehydration.

b. Blocked blood flow to or from kidney may be due to tumor, stone, or irregular heart rhythms.

c. Nephritis or urinary infection.

d. After effect of disease such as congestive heart failure, diabetic neuropathy, and enlarged prostate gland in man.

e. Gastrointestinal bleeding.

f. Low blood pressure for larger period of time.

g. Increased protein in diet.

h. Radiology procedures in which radiocontrast dye is injected intravenously for a clear picture.

i. Drug toxicity with some chemotherapeutic (carboplatin, carmustine, cisplatin, methotrexate, and mitomycin) and biologic therapeutic agents (interleukin-2 and interferon-alfa), antibiotics (amphotericin B, gentamicin, and vancomycin), NASID's (ibuprofen), diuretics (furosemide), and ACE inhibitors (captopril, benazepril, and enalapril).

j. Nephrotoxicity after herbal medicine intake.

Reason of nephrotoxicity after herbal medicine intake may be addition of toxins during careless preparation, addition of adulterants, heavy metals, and some pharmaceutical products intentionally to reduce cost or increase efficacy [4].

Herbs such as *Tripterygium wilfordii* Hook (thunder god vine) contain diterpenoid epoxide, which induces apoptosis causing kidney damage. *Averrhoa carambola* L. (star fruit) contains oxalate in high quantity, which can cause acute nephropathy. *Guaiacum officinale* L. (rough bark) and *Arctostaphylos uva-ursi* (cranberry) increase stone formation. *Aristolochia fangchi* causes well-known aristolochic acid nephropathy. *Callilepis laureola* DC (Impila) inhibits mitochondrial ATP synthesis. *Uncaria tomentosa* wild DC (Peruvian's Cat Claw) causes acute allergic interstitial nephritis. Studies are being conducted on *Salix alba* L. (willow bark) analgesic nephropathy induction. *Ephedra sinica* Stapf (Chinese ephedra) affects renin-angiotensin-aldosterone system. *Glycyrrhiza glabra* L. (Licorice) and *Harpagophytum procumbens* DC (devil's claw) inhibit renal transport processes [5].

3.2. Hepatotoxicity

Hepatotoxicity, termed after two Greek words *Hepar* and *Toxicon* meaning liver and poison, respectively, may be defined as liver damage due to chemical, drug, herb, or dietary supplement. The damage can be noticed by stomach pain, nausea, vomiting, change in urine and stool color, jaundice, rash, frequent tiredness, weakness, fatigue, and fever. Laboratory tests include some liver function tests, which are conducted on blood samples for the detection of hepatotoxicity. These tests are comprised of alanine transaminase test (normal range 7–55 U/l), aspartate transaminase test (normal range 8–48 U/l), alkaline phosphatase test (normal range 45–115 U/l), albumin test (normal range 3.5–5.0 g/dl), and bilirubin test (normal range 0.1–1.2 mg/dl). Increased levels of ALT, AST, ALP and bilirubin and decreased level of albumin indicate liver damage. In pregnancy, ALP levels are also increased.

Causes of liver damage are both by hepatocellular and by extracellular mechanisms. Some of these mechanisms are mentioned below:

Hepatocyte disruption: When drug binds to intracellular proteins covalently, it may lower ATP level causing actin disruption, which in turn causes bleb and rupture of membrane.

Transport protein disruption: Drugs affecting transport proteins at canalicular membrane may disturb flow of bile juice. This interruption in certain processes and transport prevents bilirubin excretion leading to cholestasis.

T-cell activation: When a drug binds covalently to P-450 enzyme, it acts as immunogen and activates T cells and cytokines resulting in complicated immune response.

Hepatocyte apoptosis: Sometimes apoptotic pathway is activated due to tumor necrosis and α-receptor of F triggering flow of intercellular caspases, which leads to programmed cell death.

Disruption of mitochondria: Some drugs suppress mitochondrial function by effecting on β-oxidation energy production and synthesis of nicotine amide adenine dinucleotide. A second effect is on flavin dinucleotide inhibiting ATP production.

Injury of bile duct: Some toxic metabolites from liver can injure epithelium of bile duct.

Drug toxicity mechanisms: Drugs are major cause of hepatotoxicity. Almost nine hundred drugs, toxins, and herbs are reported for hepatotoxicity. There are two kinds of drug reactions, first

is reaction directly affecting the liver termed as intrinsic drug reactions, and other is reaction mediating to immune response termed as idiosyncratic drug reactions. In the first category, drug itself or its metabolite produces a dose-related injury, for example, paracetamol and carbon tetrachloride. In second category, hypersensitivity reactions, for example, phenytoin reaction, cause fever, rash, eosinophilia for a short period of time and immunoallergic or metabolic idiosyncratic reaction due to indirect drug reaction. The second type of reactions the response rate is variable, for example, halothane.

Drug interaction mechanisms: Some drugs when taken simultaneously react together and cause liver damage. For example, tylenol can be hepatotoxic in combination with INH, isotamine, laniazid, and nydrazid [6].

Discussing about hepatotoxicity caused by herbal medicine intake, mostly the incident rates are still to be reported. The severity of toxicity is widely variable between mild hepatitis to acute hepatic failure. The scoring system for allopathic medicines can be assessed but is not suitable for herbal medicines and needs validation. Many Ayurvedic and Chinese herbal medicines are reported to cause hepatotoxicity. Major hepatotoxic herbs are *Cimicifuga racemosa* (black cohosh), *Larrea tridentata* (chaparral), *Teucrium chamaedrys* (germander), *Scutellaria lateriflora* (American skullcap), and *Scutellaria baicalensis* (Chinese skullcap), etc.[7].

3.3. Cardiotoxicity

Cardiotoxicity is a term used for damage to heart or altering heart functions. It is a state in which there is alteration in electrophysiological function of heart or cardiac muscle damage, which weakens the heart causing inefficient pumping and circulation of blood. This can be detected by symptoms such as dry, non-productive cough, inflammation of ankles, hand, feet, and neck veins; irregular heartbeat; tachycardia; cardiomegaly; weakness; vertigo, etc.

Some common tests for finding cardiotoxicity include physical examination of heart through stethoscope to check the sounds of heartbeat, chest X-ray to check the size of the heart, echocardiogram imaging test using ultrasound, electrocardiogram (ECG) to measure the electrical activity of heart, multi-gated acquisition scan (MUGA) by injecting a radiotracer into veins to check pumping and function of blood vessels to heart, and troponin blood tests. Troponins are proteins of heart muscles released by dying heart cells into blood stream.

These tests may show results positive for cardiotoxicity due to a number of cardiac events including changes in blood pressure, thrombosis, arrhythmias, inflammation of myocardium, and pericardium leading to cardiac arrest or failure. Cardiotoxic agents include chemotherapeutic drugs of anthracycline class, alkylating agents such as cyclophosphamide, cisplatin, chlormethine, mitomycin, etc. Some other agents such as paclitaxel, etoposide, fluorouracil, asparaginase, tretinoin, pentostatin may cause cardiotoxicity. This may be increased due to cumulative dose, rate, and schedule of administration, history of preexisting cardiovascular problems, and disturbed balance of cardiac electrolytes [8].

Herbal medicines having direct effect on heart include medicine prepared from plants such as *Digitalis purpurea* (digitalis), *Catharanthus roseus* (vinca), *Aconitum napellus* (monk's hood),

Atropa belladonna (deadly nightshade), *Ephedra distachya* (sea grape), *Mandragora officinarum* (mandrake), *Glycyrrhiza glabra* (licorice), etc. [9].

3.4. Neurotoxicity

Neurotoxicity is a term used for a state in which there is a physical brain damage due to exposure to neurotoxin, a substance that disrupts or kills neurons, and in turn alters the activity of nervous system. Signs and symptoms of this type of toxicity are anxiety, depression, limb weakness and numbness, impaired vision, headache, sexual dysfunction and behavioral changes. The reasons may be chemotherapy, radiation therapy, drug abuse, organ transplants, exposure to heavy metals, some food additives, pesticides, cosmetics, cleaning solvents and naturally occurring substances.

The nervous system comprises of central nervous system (CNS) and peripheral nervous system (PNS). CNS consists of brain and spinal cord, and cerebellum is a part of brain primarily affected by neurotoxic substances. Cerebellum is responsible for processing information to conduct muscle activities and maintain body posture. The damaged cerebellum produces altered reflexes, unsteady walk, loss of body control, and confusion. PNS is a network of cranial and spinal nerves emerging from CNS to all parts of body. The system consists of myelinated neurons with layers of Schwann cells, which act as electrical insulator. By the damage of these nerves, the electrical signals are interrupted. Another part of PNS is autonomic nervous system (ANS), which functions without conscious effort. The movements such as cardiovascular, respiratory, gastrointestinal, and endocrine systems are under the control of this system. The toxicity of this system is most dangerous and it results in loss of activities function, retention of urine and stool, impotence, paralysis and impotence [10].

Some common plants used as herbal medicines have potential neurotoxic effects. Among them are *Papaver somniferum* (opium), *Catharanthus roseus* (vinca), *Datura stramonium* (thorn apple), *Atropa belladonna* (deadly nightshade), *Hyoscyamus niger* (henbane), *Cannabis indica* (marijuana), *Conium maculatum* (hemlock), *Coscinium fenestratum* (yellow vine) [11], and *Brugmansia* species (angel's trumpet) [12].

3.5. Skin toxicity

Cutaneous toxicity is a term used for an evident adverse effect such as skin irritation, inflammation, or rashes of epidermal growth factor receptor caused by exposure to a plant, chemical, or environmental factor.

Skin is the largest body organ and a protective barrier comprising of a layer of dead cells and several layers of living cells. When an irritating substance reaches these living cells, these sensitive cells respond by inflammation or dermatitis. Inflammation has four parts, which include redness, pain, heat, and swelling. The skin toxicity is easiest to detect as the reaction is immediately observed.

The most common test for detecting cutaneous toxicity is patch test. In this, the skin is exposed to a small amount of diluted substance in patches and observing the reaction. The most

common source of skin toxicity is food and cosmetics, and others are medicated lotions, balms, creams, inhalers and essential oils. A variety of herbal material is available in all of the above-mentioned cosmetics and medicated products. Types of skin sensitization reactions include the following:

Primary irritant dermatitis: It is a direct irritation of skin with symptoms such as redness, itching, pain, blusters, peeling, or open wounds. Primary irritant dermatitis may be caused by plants such as *Cannabis sativa* (weed oil), *Dieffenbachia amoena* (dumb canes), *Asclepias syriaca* (milk weed), *Narcissus pseudonarcissus* (daffodils), *Digitalis purpurea* (foxglove), *Ricinus communis* (castor bean), *Tulipa gesneriana* (tulip bulb), *Primula veris* (cowslip), *Hevea brasiliensis* (rubber tree), *Ficus carica* (fig tree sap), *Ranunculus acris* (butter cup), etc. Common foods such as *Pastinaca sativa* (parsnip), *Solanum lycopersicum* (tomatoes), *Daucus carota* (carrot), *Cucumis sativus* (cucumber), *Brassica rapa* (turnip), *Petroselinum crispum* (parsley), *Apium graveolens* (celery) and *Agaricus bisporus* (mushrooms) also can cause primary irritant dermatitis.

Allergic contact dermatitis: It is a true allergic response and is varied from individual to individual. *Toxicodendron diversilobum* (poison oak) and *Toxicodendron rydbergii* (poison ivy) are the most common plants producing allergic contact dermatitis. Others include *Hedera helix* (English ivy), *Toxicodendron vernix* (poison sumac), *Dendranthema grandiflorum* (chrysanthemum), *Narcissus pseudonarcissus* (daffodils), *Tulipa gesneriana* (tulip bulb), *Marchantiophyta species* (liverwort), *Primula vulgaris* (prime rose), *Flavoparmelia caperata* (lichens), *Pinus sabiniana* (pine), *Cedrus deodara* (cedar), *Anacardium occidentale* (cashew), *Apium graveolens* (celery), *Allium cepa* (onions), and *Allium sativum* (garlic).

Photosensitization dermatitis: It is cutaneous toxic response caused by exposure to sunlight when a photosensitizer (compound sensitive to sunlight) is present in body and can be detected by sunburn-like reactions in non-pigmented areas. Plants such as *Tetradymia species* (horse brushes), *Hypericum species* (St John's wort), *Tribulus terrestris* (goats head), *Agave lechuguilla* (lechuguilla), *Bassia scoparia* (kochia), and *Lantana camara* (lantana) cause photosensitization dermatitis.

There is another type of phototoxic photosensitization caused by contact of some plants. When a photoactive chemical produced by plants comes in skin contact, absorbed and activated by sunlight, this type of reaction occurs. The intensity varies depending upon time and amount of exposure. Plants such as *Ficus carica* (figs), *Anethum graveolens* (dill), *Brassica alba* (mustard), *Petroselinum crispum* (parsley), *Citrus aurantifolia* (lime), *Daucus carota* (carrots), *Ranunculus acris* (butter cup), *Hypericum perforatum* (Klamath weed), and *Apium graveolens* (celery) with pink rot are reported to produce contact photosensitization [13].

4. Reported cases of toxicity using herbal medicine solely

Although a large number of herbal toxicity cases are not reported, still there are many reported cases. Some of such cases are listed in **Table 1**.

No.	Herb	Toxicity reported	Indication	Reference
1.	*Stephaniae sinica* *Vernacular* name: Anshu Ling *Jin Bu Huan*	Acute hepatitis	Insomnia	[14]
2.	*Larrea tridentata* *Vernacular* name: Chaparral	Hepatic failure Hepatorenal syndrome encephalopathy	Liver detoxification	[14]
3.	*Ephedra sinica* *Vernacular* name: Ma Huang	Tachycardia, difficulty in respiration, insomnia	Nutritional supplement	[15]
4.	*Scutellaria baicalensis* *Vernacular* name: Chinese skull cup; Huang Qin	Acute drug-induced liver injury	Arthritis	[16]
5.	*Panax ginseng* *Vernacular* name: Renshen Yangrong Tang	Chronic renal failure	Anorexia and hypoproteinemia	[17]
6.	*Vaccinium macrocarpon* Common name: Cranberry	Nephrolithiasis	Dietary supplement	[18]
7.	*Salix daphnoides* *Vernacular* name: Willow bark	Renal dysfunction	Analgesic anti-rheumatic	[19]
8.	*Pausinystalia johimbe* *Vernacular* name: johimbe	Progressive renal failure and proteinuria	Male impotence	[20]
9.	*Aconitum napellus* *Vernacular* name: Aconite, monk's hood	Ventricular arrhythmia	Pain	[21]
10.	*Tripterygium wilfordii* Hook F *Vernacular* name: Thunder god vine	Renal and cardiotoxicity	Arthritis	[22]
11.	*Cimicifuga racemosa* *Vernacular* name: Black cohosh	Acute hepatitis	Menopausal symptoms	[23]
12.	*Piper methysticum* *Vernacular* name: Kava kava	Acute liver failure	Tranquillizer	[24]
13.	*Valeriana officinalis Vernacular* name: Valerian	Liver toxicity, neurotoxicity	Sedative	[25]

Table 1. Some of the documented herbal toxicity cases.

5. Herb-drug interactions

Natural products are a mixture of phyto-constituents unlike the conventional drugs. Usually, the quantity and quality of the bioactive substance from the herbs vary depending on the part of the plant used, environmental factors, method of collection and storage conditions. The use of herbs as medicine is getting popular; hence, there is a need to address and review the interaction between the herb and the drug. Certain herbal supplements may cause dangerous side effects when taken with prescription drugs. Furthermore, the complex nature of a natural

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product adds to the complexity of determination of herb-drug interactions. There are no standards for herbal products prescribed/regulated by the FDA [26]. Currently, there are limited reports of adverse herb-drug reactions. The available reports are either individual case reports giving the details of the specific case studied or the suspected interaction or clinical trials, in which the drugs and herbs are combined and are closely monitored. Data concerning the drug-herb interaction are usually unavailable as there is lack of information about nature of the herbal product and their complex reactions. Sometimes the literature available can be confusing due to lack of clarity or even contradictory. This is due to the way these adverse interactions are reported. The data received by experimentation and pharmacodynamics studies may give indications of potential interactions.

Herb-drug interactions can be either pharmacodynamic or pharmacokinetic in nature. When the constituents have synergistic or antagonist activity in relation to the drug, it is termed as pharmacodynamics, which results in concentration-dependent activity, while alteration in ADME of the drug by herbal products results in pharmacokinetic interactions [27].

The Med Watch system is used by the FDA to report adverse reactions for conventional drugs as well as dietary supplements. The complex nature of the herbs creates complications while determining the herb-drug interactions.

A method for the evaluation of herb-drug interactions has been developed for determining the reliability of the case reports on drug-herb interactions. A 10-point scale has been used for detecting the probability of drug-herb interaction. This method consists of ten items and each item being allotted 01 point. The interactions are interpreted as 8–10 points' likely, 4–7 points possible, or 0–3 points unevaluable. A total of 320,860 adverse events were reported to the system in 2002 [28].

The following are the evaluating parameters for determining the probability of herb-drug interactions (1 point is allotted per item) [29] Some herb-drug interactions are listed in (**Table 2**):

a. Adequate patient history.

b. Concurrent diseases, conditions, or other medications associated with adverse event.

c. Concomitant medications are documented.

d. Description of interactors is adequate.

e. Obvious alternate explanations have been excluded.

f. Chronology is complete.

g. Time sequence of drug administration to adverse event is reasonable.

h. Adverse event is adequately described.

i. Event ceases upon stopping drug.

j. Event recurs upon re-challenge.

No.	Herb	Allopathic drug	Indications	Model	Reference
1.	*Hypericum perforatum*	Alprazolam	A twofold decrease in the AUC for alprazolam plasma concentration vs time and a twofold increase in alprazolam clearance. Shortening of alprazolam. elimination half-life.	Clinical study	[30]
2.	*Catha edulis*	Ampicillin	Significantly reduced the bioavailability of orally. administered ampicillin.	Clinical study	[31]
3.	*Piper Methysticum*	Caffeine	Myoglobinuria, rhabdomyolysis, severe muscle pain, dark urine, and elevated creatine kinase.	Case study	[32]
4.	*Ginkgo biloba*	Sodium valproate	3–4 seizures within 2 weeks.	Case study	[33]
5.	*Panax ginseng*	Phenelzine	Headache, insomnia, tremulousness.	Case study	[34]
6.	*Zingiber officinalis*	Metronidazole	Absorption and plasma half-life were significantly increased, significantly decreased the elimination rate constant and clearance of metronidazole.	Animal study	[35]
7.	*Cimicifuga racemosa*	Atorvastatin	It may potently inhibit human cytochrome (CYP) 3A4, which may result in increase of atorvastatin levels, causing an elevation of liver enzymes.	Case study	[36]
8.	*Scutellariae radix*	Losartan	The metabolic activities of losartan were decreased to 71%.	Clinical study	[37]
9.	*Camellia sinensis*	Folic acid	Results in decreased bioavailability of folic acid.	Clinical studies	[38]
10.	*Allium sativum*	Saquinavir	Reduced plasma. saquinavir concentration.	Clinical study	[39]
11.	*Glycyrrhiza glabra*	Anti-hypertensives	Patients with essential HT are more sensitive to the inhibition of beta-HSD by liquorice. Symptoms were more in women than men.	Clinical study	[40]
12.	*Papain*	Warfarin	Skin, urinary, GIT. bleeding.	Case study	[41]
13.	*Betula alba*	Warfarin	GI bleeding and a doubled prothrombin time.	Case study	[42]
14.	*Evolvulus alsinoides*	Phenytoin	Loss of seizure control.	Case study	[43]
15.	*Banisteriopsis caapi*	Fluoxetine	Tremors, shivering, sweating, severe nausea, and vomiting.	Case study	[44]

Table 2. Some of the documented herb-drug interactions.

Although one or two reports may not guarantee an absolute contraindication to combinations of herbal and prescription therapies, certain precautions have to be taken while collecting the medical history of patients during counseling sessions so as to obtain this information regarding the use of drugs and herbs in combination by the patient. Herbal drugs should be prescribed with caution in case of elderly patients, pregnant women, patients suffering from

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liver and kidney impairment and patients who have undergone organ transplant. The healthcare professionals can monitor the use of herbal medicines, especially when the patients are taking them along with the prescribed medicine. The patient has to be counseled with enough information about signs and symptoms of herb-drug interactions such that they are able to recognize any adverse reaction whenever it occurs. The patient should be advised to have a gap of 1–2 h or several hours between the intake of herb and drug.

Evidence-based research should be encouraged to document the data regarding the positive and/or negative effects of the use of herb and drugs in combination. Furthermore, it would be of help to if an internationally accessible database documenting the herb-drug interaction would be available.

6. Possible ways to reduce toxic effects of herbal medicines

Natural substances are the best healers, but according to Paracelsus (1493–1541), all substances are poison and that's only the correct dose, which make them a remedy. There are some rules mentioned in the literature, which can be summarized as follows:

a. All herbal medicines should not be considered safe unless prescribed by registered herbalist.

b. Label of herbal product must be checked for seal of regulatory authority and expiry date.

c. If consuming herbal medicine with allopathic medicines, then inform your doctor.

d. Avoid use of herbal products along with drugs having narrow therapeutic index such as warfarin, digoxin, cyclosporine, theophylline.

e. Avoid using herbal products containing heavy metals such as arsenic, lead, mercury.

f. If female user is pregnant or nursing mother, then caution taking herbal medicines such as black cohosh, chamomile, Dong Quai root, feverfew, ginger, kava kava, and St. John's wort.

g. Overuse of herbal medicine intake should be avoided and dosing instructions must be followed.

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References

[1] Barrueto F, Tarabar A (2016). Medscape. Herb Poisoning [Internet]. http://emedicine.medscape.com/article/817427-overview (accessed 04/13/2016).

[2] Jennifer K. (2002). Medicine Management in Nursing Times, Toxicity and Adverse Effects of Herbal Complementary Therapy [Internet]. http://www.nursingtimes.net/clinical-subjects/medicine-management/toxicity-and-adverse-effects-of-herbal-complementary-therapy/197707.fullarticle (accessed 4/13/2016).

[3] Barrett B, Keifer D, Rabago D. (1999). Assessing the risks and benefits of herbal medicine: an overview of scientific evidence. Altern Ther. 5(4): 40–49.

[4] Asif M. (2012). A brief study of toxic effects of some medicinal herbs on kidney. Adv Biomed Res. 1: 44. doi:10.4103/2277-9175.100144

[5] Allard T, Wenner T, Greten HJ, Efferth T. (2013). Mechanisms of herb-induced nephrotoxicity. Curr Med Chem. 20(22): 2812–9.

[6] Bunchorntavakul C, Rddey KR. (2013). Review article: herbal and dietary supplement hepatotoxicity. Aliment Pharmacol Ther. 37(1): 3–17.

[7] Andrade RJ, Robles M, Fernández-Castañer A, López-Ortega S, López-Vega MC, Lucena MI. (2007). Assessment of drug-induced hepatotoxicity in clinical practice: a challenge for gastroenterologists. World J Gastroenterol. 13(3): 329–40. doi:10.3748/wjg.v13.i3.329

[8] Pai VB, Nahata MC. (2000). Cardiotoxicity of chemotherapeutic agents: incidence, treatment and prevention. Drug Saf. 22(4): 263–302.

[9] Maffè S, Paffoni P, Laura Colombo M, Davanzo F, Dellavesa P, Cucchi L, Zenone F, Paino AM, Franchetti Pardo N, Bergamasco L, Signorotti F, Parravicini U. (2013). Herbs and cardiotoxic effects. G Ital Cardiol. (Rome) 14(6): 445–55. doi:10.1714/1280.14158

[10] Nielsen E, Brant J. (2002). Chemotherapy-induced neurotoxicity: assessment and interventions for patients at risk. Am J Nurs. 102: 16–19.

[11] Wattanathorn J, Uabundit N, Itarat W, Mucimapura S, Laopatarakasem P, Sripanidkulchai B. (2006). Neurotoxicity of Coscinium fenestratum stem, a medicinal plant used in traditional medicine. Food Chem Toxicol. l44(8): 1327–33.

[12] Parez BE, Rodriquez OR, Sanchez VMC. (2012). Toxic plants: Brugmansia (floripondio) neurotoxicity. Arch Med Urg Mex. 4(3): 119–124.

[13] Cutaneous Toxicity: Toxic Effects on Skin. 1993. Extoxnet. http://pmep.cce.cornell.edu/profiles/extoxnet/TIB/cutaneous-tox.html (accessed 5/2/2016).

[14] Haller CA, Dyer JE, Ko R, Olson KR. (2002). Making a diagnosis of herbal-related toxic hepatitis. West J Med. 176(1): 39–44. PMCID: PMC1071652.

[15] Gurley BJ, Gardner SF, White LM, Wang PL. (1998). Ephedrine pharmacokinetics after the ingestion of nutritional supplements containing Ephedra sinica (Ma huang). Ther Drug Monit. 20(4): 439–45.

[16] Yang L, Aronsohn A, Hart J, Jensen D. (2012). Herbal hepatoxicity from Chinese skullcap: a case report. World J Hepatol. 4(7): 231–3. doi:10.4254/wjh.v4.i7.231

[17] Wei L, Chen B, Ye R, Li H. (1999). Treatment of complications due to peritoneal dialysis for chronic renal failure with traditional Chinese medicine. J Tradit Chin Med. 19: 3–9.

[18] Terris MK, Issa MM, Tacker JR. (2001). Dietary supplementation with cranberry concentrate tablets may increase the risk of nephrolithiasis. Urology 57: 26–29.

[19] Schmid B, Kotter I, Heide L. (2001). Pharmacokinetics of salicin after oral administration of a standardised willow bark extract. Eur J Clin Pharmacol. 57: 387–391.

[20] Sandler B, Aronson P. (1993). Yohimbine-induced cutaneous drug eruption, progressive renal failure, and lupus-like syndrome. Urology 41: 343–345.

[21] Sheth S, Tan EC, Tan HH, Tay L. (2015). Herb-induced cardiotoxicity from accidental aconitine overdose. Singapore Med J. 56(7): e116–9. doi:10.11622/smedj.2015114

[22] Chou WC, Wu CC, Yang PC, Lee YT. (1995). Hypovolemic shock and mortality after ingestion of Tripterygium wilfordii hook F: a case report. Int J Cardiol. 49: 173–177.

[23] Chow ECY, Teo M, Ring JA, Chen JW. (2008) Liver failure associated with the use of black cohosh for menopausal symptoms. Med J Aust. 188(7): 420–422.

[24] Gow PJ, Connelly NJ, Hill RL, Crowley P, Angus PW. (2003). Fatal fulminant hepatic failure induced by a natural therapy containing kava. Med J Aust. 178(9): 442–3.

[25] Willey LB, Mady SP, Cobaugh DJ, Wax PM. (1995). Valerian overdose: a case report. Vet Hum Toxicol. 37(4): 364–5.

[26] Office of Inspector General, Department of Health and Human Services. (2001). Adverse Event Reporting for Dietary Supplements: An Inadequate Safety Valve. http://oig.hhs.gov/oei/reports/oei-01-00-00180.pdf (accessed on 5/2/2016)

[27] Mary L, Chavez ML, Jordan MA, Chave PI. (2006). Evidence-based drug-herbal interactions. Life Sci. 78: 2146–57.

[28] Center for Drug Evaluation and Research (2002). Report to the Nation. Improving Public Health through Human Drugs. U.S. Department of Health and Human Services. Food and Drug Administration Center for Drug Evaluation and Research. http://oig.hhs.gov/reading/workplan/2002/phs.pdf(accessed on 5/2/2016)

[29] Fugh-Berman A, Ernst E. (2001). Herb-drug interactions: review and assessment of report reliability. Br J Clin Pharmacol. 52(5): 587–95.

[30] Markowitz JS, Donovan JL, De Vane CL, Taylor RM, Ruan Y, Wang JS, et al. (2003). Effect of St John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. JAMA. 290: 1500–4.

[31] Charles OE. (2011). Drug-drug and herb-drug interactions—a comment. JORIND. 9(1): 47.

[32] Donadio V, Bonsi P, Zele I, Monari L, Liguori R, Vetrugno R, et al. (2000). Myoglobinuria after ingestion of extracts of Guarana, Ginkgo biloba and kava. Neurol Sci. 21: 124.

[33] Granger AS. (2001). Ginkgo biloba precipitating epileptic seizures. Age Ageing. 30: 523–5.

[34] Shader RI, Greenblatt DJ. (1985). Phenelzine and the dream machine-ramblings and reflections. J Clin Psychopharmacol. 5(2): 65.

[35] Okonta JM, Uboh M, Obonga WO. (2008). Herb-drug interaction: a case study of effect of ginger on the pharmacokinetic of metronidazole in rabbit. Ind J Pharm Sci. 70(2): 230–2.

[36] Patel NM, Derkits RM. (2007). Possible increase in liver enzymes secondary to atorvastatin and black cohosh administration. J Pharm Pract. 20(4): 341–6.

[37] So JY, Joo YC, Kyoung SL, Kyu PK, Jae WK, Bo HK, Jang HH, et al. (2009). Effects of Angelicae tenuissima radix, Angelicae dahuricae radix and Scutellariae radix extracts on cytochrome P450 activities in healthy volunteers. Basic Clin Pharmacol Toxicol. 105(4): 249–56.

[38] Alemdaroglu NC, Dietz U, Wolffram S, Spahn-Langguth H, Langguth P. (2008). Influence of green and black tea on folic acid pharmacokinetics in healthy volunteers: potential risk of diminished folic acid bioavailability. Biopharm Drug Dispos. 29(6): 335–48.

[39] Piscitelli SC, Burstein AH, Welden N, Gallicano KD, Falloon J. (2002). The effect of garlic supplements on the pharmacokinetics of saquinavir. Clin Infect Dis. 34: 234–8.

[40] Sigurjonsdottir HA, Manhem K, Axelson M, Wallerstedt S. (2003). Subjects with essential hypertension are more sensitive to the inhibition of beta-HSD by liquorice. J Hum Hypertens. 17: 125–31.

[41] Pérez JJ, Escate CA, Vega GJ, Ruiz AGJ, Macip NG. (1995). A probable case of warfarin overdose during anti-inflammatory therapy. Rev Invest Clin. 47: 311–3.

[42] Yip AS, Chow WH, Tai YT, Cheung KL. (1990). Adverse effect of topical methylsalicylate ointment on warfarin anticoagulation: an unrecognized potential hazard. Postgrad Med J. 66: 367–9.

http://dx.doi.org/10.5772/64468

[43] Dandekar UP, Chandra RS, Dalvi SS, Joshi MV, Gokhale PC, Sharma AV, et al. (1992). Analysis of clinically important interaction between phenytoin and shankhapushpi, an ayurvedic preparation. J Ethnopharmacol. 35: 285–8.

[44] Callaway JC, Grob CS. (1998). Ayahuasca preparations and serotonin reuptake inhibitors: a potential combination for severe adverse interactions. J Psychoactive Drugs. 30: 367–9.

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This edited book, Toxicology - New Aspects to This Scientific Conundrum, is intended to provide an overview on the different xenobiotics employed every day in our anthropogenic activities. We hope that this book will continue to meet the expectations and needs of all interested in the implications for the living species of known and new toxicants and to guide them in the future investigations.

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