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Acute and Chronic Effects of Emerging Contaminants

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Abstract Acute or chronic toxicity profiling represents one of the critical elements for scientifically reliable characterization and prioritization of potentially hazardous contaminants. The very same is true for so-called emerging contaminants, regardless of the definition used in defining various aspects of "emerging", including substances

that belong to new chemical classes, new types of use, new effects, mechanism of action, source, or exposure route. From the (eco)toxicological perspective, however, there are two essential drawbacks which prevent efficient characterization of risk posed to humans and the environment by the presence of emerging contaminants. First is related to the fact that the potential of analytical chemistry to measure contaminants currently exceeds our understanding of their potential environmental effects. Secondly, for most emerging contaminants there is currently little information regarding their potential toxicological significance in ecosystems, particularly the effects from long-term low-level environmental exposures. Based on these facts a brief overview of acute and chronic toxic effects on human and wildlife, reported for various classes of emerging contaminants, is presented in this chapter. The most demanding research unknowns, methodological drawbacks, and priorities will be highlighted, and finally, future strategies needed for efficient (eco)toxicological characterization of emerging contaminants will be suggested.

Keywords Acute and chronic toxicity · (Eco)toxicological characterization · Emerging contaminants

Abbreviations

AFOs	Animal feeding operations
ALS	Amyotrophic lateral sclerosis
ARGs	Antibiotic resistance genes
BADGE	Bisphenol A diglycidyl ether
BPA	Bisphenol A
CHE	The Collaborative on Health and the Environment
DES	Diethylstilbestrol
ELS	Early life-stages
GDS	Genotoxic disease syndrome
HAdV	Human adenoviruses
HEV	Hepatitis E virus
HPV	High Production Volume
MATC	Maximum acceptable toxicant concentration
MXR	Multixenobiotic resistance
NOAA	US National Centers for Coastal Ocean Science
OSPAR	Oslo and Paris Convention for the Protection of the Marine Environment of the North-East Atlantic
PBDEs	Polybrominated diphenyl ethers
PCNs	Polychlorinated naphthalenes
PCPs	Personal care products
PFCs	Perfluorochemicals
POPs	Persistent organic pollutants
PVC	Poly-vinyl chloride
QDs	Quantum dots
REACH	Registration, Evaluation, and Authorization of Chemicals
STP	Sewage treatment plant
US FDA	US Food and Drug Administration
USCDC	US Centers for Disease Control
USEPA	US Environmental Protection Agency
WWF	World Wide Fund

1 Introduction

Cancer, reproductive disorders, impaired neurological development, allergies – these are the types of health effects that make headlines. That puts corresponding chemicals "culprits" on the top of any list of emerging contaminants: potentially toxic substances whose effects or presence are poorly known, often because these chemicals have only begun to enter the human water or food supply. On the other hand, humans and wildlife are constantly exposed to a variety of contaminants present at low levels. These include both new chemicals, with previously unknown effects and those with well known acute (short-term exposure) human and ecological health effects. The result has been new research on emerging contaminants and an increased emphasis on methods of analyzing health effects of contaminants. The area in which several advances have recently been made is related to long-term health effects of chemical exposure. Other studies are now examining the impacts of organic compounds which may interfere with the endocrine systems of living organisms. Another active area of research is focused on how chemicals interact with each other and the natural environment. Finally, researchers are continuing to find new chemicals that bioaccumulate in the food chain. Such chemicals can be present in water at very low levels, however, they accumulate to higher concentrations in living tissue, substantially magnifying any health effects.

Three components have been usually considered to be critical for a chemical to be classified as highly hazardous contaminant: (1) persistence (structural stability resulting in long environmental half-lives); (2) lypophilicity (resulting in bioconcentration and possible biomagnification in the food chain); and (3) proven acute or chronic toxicity. However, all of these criteria need certain reconsideration – for example, continual release of some contaminants by the sewage treatment plants (STPs) give them a "pseudo-persistance" in aquatic environments; some drugs are actively transported in cells regardless of their lipid-water partition coefficients; finally, chemicals may act as indirect toxicants (such as nanoparticles or antibiotics, for example). Nevertheless, toxicity remains one of the cornerstones for scientifically reliable classification and hazard prioritization. From the (eco)toxicological perspective, however, two serious drawbacks appears to be essential in preventing efficient and reliable characterization of risk posed to humans and the environment by the presence of emerging contaminants.

Firstly, due to recent improvements in analytical chemistry, the types of chemicals that can be detected are increasing, and the limits of concentration at which they can be detected are continuously lowered. Our ability to measure contaminants currently exceeds our understanding of their potential environmental effects. Proving the link between real environmental exposure levels and acute or chronic toxic effects to humans and/or wildlife is an expensive, time-consuming, and complex research endeavor. Evaluat-

ing ecological effects of environmental contamination extends beyond observing co-occurrence of contaminants and adverse effects to documenting cause-and-effect relationships. Research to characterize cause-and-effect relationships requires documentation of contaminant uptake, modes of action, and biological endpoints. Numerous substances that act through specific or sensitive mechanisms of action (e.g., mediated by receptors or other mechanisms) may have effects on the environment or sensitive human populations at concentrations well below those previously considered to be safe. Clearly, traditional (eco)toxicological methods are not adequate to address the complexity of emerging environmental contaminants. It is a new challenge for toxicologists to effectively identify and assess the potential impact of these substances on human and ecological receptors, so that appropriate decisions can be made that balance the societal and environmental benefits and risks.

Secondly, for most emerging contaminants, there is currently little information regarding their potential toxicological significance in ecosystems, particularly effects from long-term, low-level environmental exposures. Furthermore, the fact is that we know very little about the vast majority of the chemicals we use. In the EU, more than 100 000 chemicals were reported to be on the market in 1981, which was the first and only time that the chemicals used in the EU were listed[1]. For 99% of chemicals (by volume), information on properties, uses, and risks is sketchy. Chemicals produced in high volumes (above 1000 tons per year) have been examined more closely, and there are still no data for about 21% of them. Another 65% come with insufficient data. Similar figures would be anticipated for the US and Japan (Table 1). Therefore, the raise of emerging contaminants may be only an inevitable consequence of this disproportion.

Table 1 Estimated numbers or proportions of indexed, commercially available, regulated/inventoried, and/or toxicologically characterized chemicals [172]

No. of chemicals indexed in the CAS Registry	>26 000 000
No. of commercially available chemicals	8 400 000
No. of regulated and/or inventoried chemicals	240 000
No. of chemicals marketed in the US/EU	100 000
No. of bioactive compounds in various R&D phases	>150 000
Proportion of chemicals (by volume) with known properties and risks	1%
Proportion of high volume (>1000 t) chemicals sufficiently characterized	79%
Proportion of high volume (>1000 t) chemicals insufficiently characterized	65%

[1] Public availability of data on EU high production volume chemicals, European Chemicals Bureau, Joint Research Centre, European Commission (http://ecb.jrc.it/Data-Availability-Documents/datavail.doc).

In an attempt to illustrate these critical drawbacks in this chapter we will try to present a brief overview of acute and chronic effects to human and wildlife, reported for various classes of emerging contaminants present in waste waters and aquatic environments in general. In addition, we will highlight the most demanding research unknowns, methodological drawbacks and priorities, and, finally, address future strategies needed for efficient (eco)toxicological characterization of potentially harmful substances.

2
Emerging Contaminants from (Eco)toxicological Perspective

2.1
Definition(s) – Emerging Contaminants vs. Emerging Concerns

"Emerging contaminants" can be broadly defined as any synthetic or naturally occurring chemical or any microorganism that is not commonly monitored in the environment, but has the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects. In some cases, release of emerging chemical or microbial contaminants to the environment has likely occurred for a long time, but may not have been recognized until new detection methods were developed. In other cases, synthesis of new chemicals or changes in use and disposal of existing chemicals can create new sources of emerging contaminants. Not all of these substances can accurately be described as emerging contaminants or pollutants. Some of them are found naturally in our surface waters; others are natural substances which are concentrated by anthropogenic activities; and still others are man-made chemicals that do not occur in nature. Those pollutants that are truly new, those that have just gained entry into the environment, are relatively rare in comparison to known chemicals already being released into aquatic environments, and are often confused with those whose presence has just been detected but which have long been present [1]. The term "emerging" is also used to describe not the pollutant itself, but rather a new "emerging concern", i.e. newly demonstrated toxic effect and/or mechanism of action of an old pollutant [2]. This approach is highly legitimate and is often favored among toxicologists in comparison to classifications and definitions based on chemical entities. In reality, however, scientists and regulators will have to deal with both, "emerging contaminants" and "emerging concerns", and this artificial partition is certainly not critical for principal understanding of the problem and its possible solutions.

Furthermore, once a substance is called an emerging contaminant, the longevity of its emerging contaminant status in the view of scientists and the public is largely determined by whether the biological or chemical agent of concern is persistent and/or has potentially deleterious human or eco-

toxicological effects. Alternatively, new observations or information (e.g., endocrine disruption) on contaminants (e.g., nonylphenol) can cause the reconsideration of a well known contaminant as a (re)emerging contaminant. Unfortunately, the same analytical advances which bring contaminants to the public's attention do not offer knowledge about whether the newly detected contaminant is of (eco)toxicological interest. Assessing the effects of these contaminants in the environment remains a major time- and resource-intensive challenge. Therefore, it is not surprising that, for the many thousands of chemicals being produced or already on the market and the many new microbes that are being discovered, advances in our understanding of their (eco)toxicological properties are considerably slow and lag significantly behind the public's demand for information. As a result, a contaminant may be considered for several years to be emerging. Regardless of the definition in this chapter we will cover different dimensions of "emerging", including substances that belong to new chemical classes, new types of use, new effects, mechanism of action, source, or exposure route.

3
Human vs. Ecological Health Effects

3.1
Human Health Effects – Basic Principals

Human health results from complex interactions among genes and the environment. Environmental exposures to chemical, physical, and biological agents may cause or contribute to disease in susceptible individuals. Personal lifestyle factors, such as diet, smoking, alcohol use, level of exercise, and UV exposure, often are a primary focus when considering preventable causes of disease. However, exposures to chemical contaminants at work, at home, in the outdoors, and even in utero, are increasingly recognized as important and preventable contributors to human disease [3].

Toxic effects of chemical agents are often not well understood or appreciated by healthcare providers and the general public. Some chemicals, such as asbestos, vinyl chloride, and lead, are well established as causes of human disease. There is also good evidence available to suggest increases in the incidence of some cancers, asthma, and developmental disorders, can be attributed to chemical exposure, particularly in young children. Other diseases, such as amyotrophic lateral sclerosis (ALS) or Gulf War Syndrome have been hypothesized to be associated with chemical exposures, but the evidence is limited.

The effects of chemical exposures in humans are difficult to study, because controlled human experimentation is not ethically feasible. There is limited human data obtained from accidental exposures, overdoses, or studies of work-

ers exposed occupationally. Environmental exposure studies in the general population also can be useful, though they often have limitations. Many diseases, such as cancer, may not appear until decades after an exposure has occurred, making it difficult for causal associations to be identified. Exposure assessment, a critical step in environmental epidemiologic studies, is difficult. Retrospective exposure assessment usually requires estimates and considerable judgment and is subject to significant error. An individual's exposure may change over time, and exposures to multiple chemicals occur both in the home and work environments. It is difficult for individuals to remember or even know what they have been exposed to. Furthermore, the effects of chemical exposures may vary, depending on the age of exposure (in utero, childhood, adult), the route of exposure (ingestion, inhalation, dermal), amount and duration of exposure, exposures to multiple chemicals simultaneously, and other personal susceptibility factors, including genetic variability.

Because of these challenges, most toxicity research is conducted in animal studies, which contribute important toxicological information and provide strong evidence of disease without human epidemiological studies if the mechanism of action is relevant. Many regulatory decisions to limit or ban the use of a chemical are based on animal data. Furthermore, human epidemiology studies are often conducted after an association has been hypothesized based on animal data. The same is true for most data related to human toxic effects of emerging contaminants described in this chapter.

Although there is a need for much more chemicals to be adequately characterized, a vast amount of data for human acute or chronic toxic effects of various contaminants is already available and published. What is often lacking, both for scientists and regulators, as well as for citizens, is a comprehensive and reliable tool that offers free, scientifically sound, and reliable information about contaminants hazardous to humans. Nevertheless, useful and comprehensive evidence has been recently complied within two independent sources. With the motto: "Mapping the Pollution in People", The Human Toxome Project at the Environmental Working Group in the USA [4] established a web database aimed at collecting and presenting relevant data about health effects of virtually all pollutants that enter the human body. Another source is The Collaborative on Health and the Environment (CHE) Toxicant and Disease Database [5], a searchable database that summarizes links between chemical contaminants and approximately 180 human diseases or conditions.

3.2 Ecotoxicological Aspects of Emerging Contaminants

As much as it is difficult to establish clear causal connections between contaminant(s) exposure and human health effects, it is far more difficult to do the same on the ecosystem level, with numerous species involved at different

levels of biological organization, and many environmental factors that make the interpretation of field data even more complex. Paradoxically (or not?), knowledge, expertise, and resources being invested in human health issues, outmatch multiple times those invested in the environmental health arena, explaining to a large extent the critical shortage in data needed for a sustainable management of environmental resources.

More specifically, the objective of aquatic toxicity tests with effluents or pure compounds is to estimate the "safe" or "no effect" concentration of these substances, which is defined as the concentration that will permit normal propagation of fish and other aquatic life in the receiving waters. The endpoints which have been considered in tests to determine the adverse effects of toxicants include death and survival, decreased reproduction and growth, locomotor activity, gill ventilation rate, heart rate, blood chemistry, histopathology, enzyme activity, olfactory function, etc. [6]. Since it is not feasible to detect and/or measure all of these (and other possible) effects of toxic substances on a routine basis, observations in toxicity tests generally have been limited to only a few effects, typically including mortality, growth, and reproduction.

Acute lethality is an obvious and easily observed effect which accounts for its wide use in the early period of evaluation of the toxicity of pure compounds and complex effluents. The results of these tests were usually expressed as the concentration lethal to 50% of the test organisms (LC50) over relatively short exposure periods (one-to-four days).

As exposure periods of acute tests were lengthened, the LC50 and lethal threshold concentration were observed to decline for many compounds. By lengthening the tests to include one or more complete life cycles and observing the more subtle effects of the toxicants, such as a reduction in growth and reproduction, more accurate direct estimates of the threshold or safe concentration of the toxicant could be obtained. However, laboratory life-cycle tests may not accurately estimate the "safe" concentration of toxicants, because they are conducted with a limited number of species under highly controlled, steady-state conditions, and the results do not include the effects of the stresses to which the organisms would ordinarily be exposed in the natural environment.

An early published account of a full life-cycle fish toxicity test was that of Mount and Stephan back in 1967 [7]. In this study, fathead minnows, *Pimephales promelas*, were exposed to a graded series of pesticide concentrations throughout their life-cycle, and the effects of the toxicant on survival, growth, and reproduction were measured and evaluated. This work was soon followed by full life-cycle tests using other toxicants and fish species. McKim [8] evaluated the data from 56 full life-cycle tests, 32 of which used the fathead minnow, and concluded that the embryo-larval and early juvenile life-stages were the most sensitive stages. He proposed the use of partial life-cycle toxicity tests with the early life-stages (ELS) of fish to establish water qual-

ity criteria. Macek and Sleight [9] found that exposure of critical life-stages of fish to toxicants provides estimates of chronically safe concentrations remarkably similar to those derived from full life-cycle toxicity tests. They reported that for a great majority of toxicants, the concentration which will not be acutely toxic to the most sensitive life stages is the chronically safe concentration for fish, and that the most sensitive life stages are the embryos and fry. Critical life-stage exposure was considered to be exposure of the embryos during most, preferably all, of the embryogenic (incubation) period, and exposure of the fry for 30 days post-hatch for warm water fish with embryogenic periods ranging from 1–14 days, and for 60 days post-hatch for fish with longer embryogenic periods. They concluded that in the majority of cases, the maximum acceptable toxicant concentration (MATC) could be estimated from the results of exposure of the embryos during incubation, and the larvae for 30 days post-hatch.

In a review of the literature on 173 fish full life-cycle and ELS tests performed to determine the chronically safe concentrations of a wide variety of toxicants, such as metals, pesticides, organics, inorganics, detergents, and complex effluents, Woltering [10] found that at the lowest effect concentration, significant reductions were observed in fry survival in 57%, fry growth in 36%, and egg hatchability in 19% of the tests. He also found that fry survival and growth were often equally sensitive, and concluded that the growth response could be deleted from routine application of the ELS tests. The net result would be a significant reduction in the duration and cost of screening tests with no appreciable impact on estimating MATCs for chemical hazard assessments.

Efforts to further reduce the length of partial life-cycle toxicity tests for fish without compromising their predictive value have resulted in the development of an eight-day embryo-larval survival and teratogenicity test for fish and other aquatic vertebrates [11, 12], and a seven-day larval survival and growth test [13]. The similarity of estimates of chronically safe concentrations of toxicants derived from short-term embryo-larval survival and teratogenicity tests to those derived from full life-cycle tests has been firstly demonstrated by Birge et al. [12, 14].

Since that time, most of our knowledge about acute and chronic effects of contaminants originates from the described type of ecotoxicity tests. An overview of the present knowledge related to emerging contaminants/concerns will be presented in the next section.

4 Human and Environmental Health Effects

Among many different categories of emerging contaminants, we will especially take into consideration those which, according to the state-of-the-art litera-

Table 2 Major human/environmental health concerns and priority status of the most prominent categories of emerging contaminants

Health concern	Chemical family						
	Alkyl-phenols	Bisphenol A & BADGE	Brominated dioxins & furans	Per-chlor-ate	Perfluoro-chemicals (PFCs)	Phtal-ates	Polybrom-inated di-phenyl ethers (PBDEs)
Birth defects and developmental delays	+	+		+	+	+++	++
Brain and nervous system					++	+++	+++
Cancer		+	+		+	+	+
Endocrine system	+			+++	+		
Gastrointestinal (including liver)					+		+
Hematologic (blood) system				+			
Hormone activity	+	+++			+++	+++	+++
Immune system (including sensi-tization and allergies)		++	+		+++	+++	
Kidney and renal system		+			+++		
Reproduction and fertility	+++	++	++		+++	+++	+++
Skin		+				+	
Respiratory system	+					+++	
Wildlife and environ-mental toxicity	+++	++					+
Persistent, accumulates in wildlife and/or people	++	++	+++		++	++	++
OSPAR list	√	√				√	√
Priority substance and/ or banned in the EU, USA or Canada	√					√	

Weight of evidence: + limited; ++ probable; +++ strong

ture evidence, appear to be of the highest (eco)toxicological relevance and are frequently detected in industrial and/or municipal waste: industrial chemicals (new and recently recognized), personal care products, pharmaceuticals, nonculturable biological pathogens, and, finally, nanomaterials. Instead of referring to numerous studies utilizing various in vivo and in vitro test systems in attempts to characterize toxicity of many different contaminants, what follows in the section(s) below is a brief summary describing relevance and toxic effects reported with a reasonable weight of evidence for the most prominent emerging contaminants. Basic info referring to major human health concerns, wildlife toxicity, bioaccumulation/persistency potential, and the regulatory status of those substances is presented in Table 2.

Table 2 (continued)

Health concern	Chemical family					
	Polychlorinated naphthalenes (PCNs)	Fragrances (nitro- and polycyclic musks)	Triclosan	Pharmaceuticals	Non-culturable biological pathogens	Nanomaterials
Birth defects and developmental delays				+++		
Brain and nervous system				+		+
Cancer		+		+		
Endocrine system		+	+	+++		
Gastrointestinal (including liver)	+++	+++		+	+++	
Hematologic (blood) system						
Hormone activity				+++		
Immune system (including sensitization and allergies)		+	+		+	+
Kidney and renal system						
Reproduction and fertility	+	+++	+	++		
Skin	+++	+	+	+	++	
Respiratory system				+	+++	++
Wildlife and environmental toxicity	++	+	+	++		+
Persistent, accumulates in wildlife and/or people	++	++	++	+		
OSPAR list	√	√				
Priority substance and/ or banned in the EU, USA or Canada	√		√			

Weight of evidence: + limited; ++ probable; +++ strong

4.1 Industrial Chemicals

4.1.1 Alkylphenols

Alkylphenols are widely used industrial chemicals which act as detergents or surfactants. They are added to cosmetics, paints, pesticides, detergents, and cleaning products. Alkylphenols have been recently detected in surface waters contaminated with urban runoff and in wastewater effluents [15, 16] and have been measured in air samples. One study found that newer homes, espe-

cially those with poly-vinyl chloride (PVC) materials, have more alkylphenol residues than older houses or outdoor air [17]. As a group they are highly toxic to aquatic organisms. Dozens of recent studies have documented the in vitro and in vivo estrogenic activity of alkylphenols in human cell lines and animals [18–20]. Recent study by McClusky and colleagues [21] revealed harmful effects of *p*-nonylphenol exposure to spermatogenic cycle in male rats. Similar estrogenic activities of alkylphenols have been reported for aquatic organisms, including a recent example of the reduction of reproductive competence of male fathead minnow upon exposure to environmentally relevant mixtures of alkylphenolethoxylates [22]. Further supported by their persistency in aquatic environments and bioaccumulation potential, alkylphenols are put on the OSPAR list of possible substances of concern and included in the list of priority substances in the EU water policy.

4.1.2 Bisphenol A and Bisphenol A Diglycidyl Ether

In use since the 1950's, bisphenol A (BPA) is a building block for polycarbonate plastic and epoxy resins. BPA and its derivative, bisphenol A diglycidyl ether (BADGE), are found in many everyday products, such as the lining of metal food and drink cans, plastic baby bottles, pacifiers, and baby toys, dental sealants, computers, cell phones, hard plastic water bottles (such as Nalgene), paints, adhesives, enamels, varnishes, CDs and DVDs, and certain microwavable or reusable food and drink containers. These compounds have been shown to leach into food and water from containers – particularly after heating or as plastic ages.

BPA is a hormone-mimicking chemical that can disrupt the endocrine system at very low concentrations. More than a hundred animal studies have linked low doses of bisphenol A to a variety of adverse health effects, such as reduced sperm count, impaired immune system functioning, increases in prostate tumor proliferation, altered prostate and uterus development, insulin resistance, alteration of brain chemistry, early puberty, and behavioral changes [23–36]. Significantly, many of the studies showing adverse effects are at levels many times lower than what the US Environmental Protection Agency (USEPA) considers safe (50 μg/kg/day).

For BADGE, a bisphenol A derivative used to make epoxy resins and in a variety of industrial, engineering, and construction applications, the major pathway for human exposure is through chemical leaching from the linings of food and drink cans. BADGE is also found in some dental sealants [37].

Some basic toxicological testing has been done on BADGE, but the compound has not been extensively studied. One of the most important toxicological questions is whether BADGE breaks down into bisphenol A in the human body. Based on urinary levels of BPA in workers exposed to BADGE versus unexposed controls, researchers concluded that BADGE breaks down

into BPA in the body [38]. However, other research has suggested that there is no such biotransformation [39]. In the human body, BADGE appears in a hydrolysis product known as BADGE 40-H [40]. BADGE is quickly metabolized by the body (within a day or so), therefore body burden levels represent recent exposures.

Considering that its sister chemical, bisphenol A, has a non-monotonic dose response curve, showing nonintuitive patterns of toxicity, it would be difficult to make a final assessment on the toxicity of BADGE without more detailed study. There is some evidence that BADGE is a rodent carcinogen, but data for humans is lacking [41, 42]. Workers using epoxy resin in the construction industry have shown BADGE to be a contact allergen [43]. Males exposed to BADGE through spraying epoxy resin have associated depressed gonadotrophic hormones [38]. A study of BADGE given to pregnant rabbits found that at the lowest dose tested (30 mg/kg/day for days 6 to 18 of gestation) BADGE affected pregnancy ability and the sex ratio of their litters [39]. An in vitro study found that BADGE can induce time and dose-dependent morphological changes and cell detachment from the substratum and can inhibit cell proliferation [44]. Another study found that a BADGE derivative (BADGE.2HCl) can act as an androgen antagonist in in vitro systems [45].

4.1.3
Brominated Dioxins and Furans

Brominated dioxins and furans are toxic, persistent, bioaccumulative, and lipophilic ("fat-loving"). Along with dioxins, furans are pollutants produced during PVC plastic production, industrial bleaching, and incineration. They build up in human tissues, are stored in fatty tissues and fluid, such as breast milk, and can be passed on to fetuses and infants during pregnancy and lactation. Brominated dioxins and furans are formed unintentionally, either from incineration of wastes which include consumer products infused with brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), or as trace contaminants in mixtures of bromine-containing chemicals. Primary (eco)toxicological concern for brominated dioxins and furans is their dioxin-like activity, meaning that they cause birth defects in animals and otherwise disrupt reproductive development and the immune and hormone systems [46–49]. They add to the total dioxin body burden of people, which are near levels where adverse health effects may be occurring in the general population [50].

4.1.4
Perchlorate

The vast majority of perchlorate manufactured is used to make solid rocket and missile fuel, while smaller amounts of perchlorate are also used to make

firework and road flares. Perchlorate is also a contaminant of certain types of fertilizer which were widely used in the early part of the 20th century, but are in limited use today [51]. According to the analysis of the USEPA's latest data, perchlorate is known to be contaminating at least 160 public drinking water systems in 26 US states [52]. Tests of almost 3000 human urine and breast milk samples, along with tests of more than 1000 fruit, vegetable, cow's milk, beer, and wine samples, reveal that perchlorate exposure in the population is pervasive. Every urine sample tested showed some level of perchlorate contamination, and almost 70% of the fruit and beverage samples tested have had detectable perchlorate [52–60].

Critical toxic effect of perchlorate is inhibition of the thyroid's ability to take up the nutrient iodide, which is a key building block for thyroid hormones. If the thyroid gland does not have enough iodide for a sufficient period of time, body's thyroid hormone levels will eventually drop. Hypothyroidism (low thyroid hormone levels) in adults can cause fatigue, depression, anxiety, unexplained weight gain, hair loss, and low libido. More serious, however, are the effects of thyroid hormone disruption in the developing fetus and child. Small changes in maternal thyroid hormone levels during pregnancy have been associated with reduced IQs in children [61, 62]. A recent epidemiological study by the US Centers for Disease Control (USCDC) shows that perchlorate exposures commonly found in the population can cause significant thyroid hormone disruptions in women – particularly in the population of women with lower iodine intake. Relying on a flawed industry study, the USEPA adopted a water clean-up standard for superfund sites of 24.5 ppb in 2006. Neither the USEPA nor the US Food and Drug Administration (USFDA) have taken any action to address the problem of widespread contamination in food.

Considering animal studies, perchlorate was first discovered to affect the thyroid in the 1950s, but it wasn't until the early 1990s that scientists began to conduct studies that involved feeding low doses of perchlorate to animals and looking for adverse effects. In 1995 the USEPA found that laboratory animals developed thyroid disorders after two weeks of drinking perchlorate-laced water. Subsequent studies found effects on brain and thyroid structure at even lower doses, and noted that rat pups born to exposed mothers were particularly like to show adverse effects [53, 54].

The USCDC conducted the first major epidemiological study on perchlorate exposure in the general population [59]. After testing urine samples of 2299 men and women from around the country for perchlorate, and comparing these findings with the levels of thyroid hormones found in the blood of these same people, the USCDC's researchers discovered that there was a statistically significant relationship between urinary perchlorate and thyroid hormone levels in the 1111 women tested. Furthermore, they found that if low iodine woman started with perchlorate exposure corresponding to 0.19 ppb in urine (the minimum level found), and then ingested enough perchlorate

through food and/or drinking water to raise their urinary perchlorate level to 2.9 ppb (the median level found), their T4 thyroid hormone levels would drop by 13 percent. Similarly, if woman's urinary perchlorate level increased to 5.2 ppb (the 75th percentile exposure), their T4 levels would drop by 16 percent. These are significant declines when one considers that recent studies have shown that the cognitive development of the fetus is impaired in mothers with even mild disruptions in thyroid hormone levels [59, 61, 62]. Women with low iodine intake and levels of TSH (a type of thyroid hormone) that were already on the edge of the normal range were found to be even more sensitive to perchlorate exposure. For these women, if they were exposed to 5 parts per billion of perchlorate via food or drinking water, the resulting hormone disruption would push them into sub-clinical hypothyroidism.

4.1.5 Perfluorochemicals

The USEPA has described perfluorochemicals (PFCs) as combining "persistence, bioaccumulation, and toxicity properties to an extraordinary degree" [63]. PFCs are industrial chemicals widely used as water, stain, and grease repellants for food wrap, carpet, furniture, and clothing. The family includes such well known name brands as Scotchgard and Teflon.

PFCs are released to the environment in air and water emissions at numerous manufacturing and processing facilities worldwide. PFCs are also likely released to the environment at countless secondary manufacturing facilities, including sites where consumer products are coated for water, stain, and grease repellency. The dominant sources of PFCs in the environment are thought to be fluorotelomer chemicals, the active ingredients in coatings of furniture, clothing, food packaging, and other products. Fluorotelomers break down in the environment and in the body to PFCs differing only in the carbon chain length and end group [64, 65]. Most PFCs are fairly mobile in water, but due to low volatility of the persistent carboxy acids and sulfonates, many do not have the potential to migrate in air far from locations of release as a manufacturing pollutant. In contrast, studies indicate that PFC telomers are relatively volatile and could migrate long distances through the atmosphere.

Fluorotelomers are a likely source of the persistent perfluorochemicals found in newborns, and in wildlife and water in areas remote from manufacturing sites and human populations. Available scientific findings to date show that PFCs widely contaminate human blood [66, 67] and persist in the body for decades [68]. They act through a broad range of toxic mechanisms of action to present potential harm to a wide range of organs (ovaries, liver, kidney, spleen, thymus, thyroid, pituitary, testis), and persist indefinitely in the environment with no known biological or environmental breakdown mechanism [69–71]. Considering their ecotoxicity the newest evidence suggests

that PFCc are able to induce and inhibit the activity of xenobiotic efflux transport proteins in marine bivalves [72].

4.1.6 Phthalates

Found within many consumer products, phthalates are industrial plasticizers that impart flexibility and resilience to plastic. They are common additives to soft plastic, especially PVC. They are present in clear food wrap, personal care products (detergents and soaps), and pesticides [73].

Phthalates are widely detected in human blood and urine samples. The latest exposure study from USCDC indicates that women are slightly more exposed than men, and younger children (ages 6–11) are more exposed than older children (ages 12–20) [74]. Exposure to phthalates occurs through direct use of cosmetics and other consumer products containing these chemicals, consumption of foods wrapped in products containing these chemicals, and through inhalation of air contaminated with these chemicals [74].

In laboratory animals, fetal exposure to phthalates causes significant developmental toxicity, especially of the male reproductive system. In adult animals, phthalates damage the reproductive organs, adrenal, liver, and kidney [75]. In utero exposure to high levels of phthalate metabolites are associated with marked differences in the reproductive systems of baby boys; the exposure levels associated with these health effects were not extreme, but rather were typical for about one-quarter of all women. Adult men with high levels of phthalates have lower sperm motility and concentration and alterations in hormone levels [76–78]. Concentrations of two phthalates in house dust are associated with asthma and rhinitis in a study of 400 children, half of whom had allergies [79].

4.1.7 Polybrominated Diphenyl Ethers

Polybrominated diphenyl ethers (PBDEs) are brominated fire retardants, intentionally added to flexible foam furniture, primarily mattresses, couches, padded chairs, pillows, carpet padding and vehicle upholstery, and to electronic products.

Studies of laboratory animals link PBDE exposure to an array of adverse health effects including thyroid hormone disruption, permanent learning and memory impairment, behavioral changes, hearing deficits, delayed puberty onset, decreased sperm count, and fetal malformations [80–82]. Research in animals shows that exposure to brominated fire retardants in utero or during infancy leads to more significant harm than exposure during adulthood, and at much lower levels [47]. PBDEs are bioaccumulative and lipophilic, and, therefore, are highly persistent in people and the environment. The chemicals

build up in the body, are stored in fatty tissues and body fluids, such as blood and breast milk, and can be passed on to fetuses and infants during pregnancy and lactation. People are primarily exposed to PBDEs in their homes, offices, and vehicles. Secondary sources are foods, primarily meat, dairy, fish, and eggs [83].

Some PBDEs were withdrawn from the US market in 2005 due to their toxicity to laboratory animals, and their detection as contaminants in humans, wildlife, house and office buildings, and common foods [84–86]. Deca (PBDE-209), the form used in electronics, continues to be used in televisions, computer monitors and other electronic products. There is widespread concern that Deca breaks down in the environment to more toxic and persistent forms.

4.1.8
Polychlorinated Naphthalenes

There are 75 possible chemical variations of polychlorinated naphthalenes (PCNs). They have been used as cable insulation, wood preservatives, engine oil additives, electroplating masking compounds, capacitors, and in dye production. Products are generally mixtures of several different PCNs. The largest source of PCNs believed to be waste incineration and disposal of items containing PCNs, although other potential sources of PCNs to the environment include sewage discharge from municipal and industrial sites leaching from hazardous waste sites. PCNs are also unwanted byproducts formed after the chlorination of drinking water [87]. They have not been used commercially in significant quantities since the 1980s.

PCNs are toxic, persistent and bioaccumulate in people and wildlife. The toxic effects of many PCNs are thought to be similar to dioxin. In humans, severe skin reactions (chloracne) and liver disease have both been reported after occupational exposure to PCNs. Other symptoms found in workers include cirrhosis of the liver, irritation of the eyes, fatigue, headache, anaemia, haematuria, impotentia, anorexia, and nausea. At least ten deaths were reported from liver toxicity. Workers exposed to PCNs also have a slightly higher risk of all cancers combined [88–90].

4.2
Personal Care Products (PCPs)

4.2.1
Fragrances – Nitromusks and Polycyclic Musks

Nitromusk and polycyclic musks are synthetic fragrances typically used in cosmetics, perfume, air fresheners, cleansing agents, detergents, and soap. Musks are also used as food additives, in cigarettes, and in fish baits. Com-

monly used musks contaminate lakes and fish in the US and Europe [91–96]. Nitromusk and polycyclic musks tend to accumulate in the fatty tissues of our bodies, and are often detected in breast milk as well as blood [96–98].

In laboratory studies, some nitromusks have been linked to cancer [99, 100]. Studies of nitromusks in people suggest that high levels of some of these chemicals are associated with reproductive and fertility problems in women [101]. Some also produce skin irritation and sensitization [102, 103].

Growing concerns about the health effects of nitromusks have led the EU to ban the use of some of these chemicals in cosmetics and personal care products. As a result, the use of polycyclic musks has increased. However, laboratory studies suggest that polycyclic musks, like nitromusks, may also affect hormone systems [104–109]. Two particular musk chemicals, a nitromusk and a polycyclic musk which both produced neurotoxic effects in laboratory animals, have been removed from the market. In the US, all musk chemicals are unregulated, and safe levels of exposure have not yet been set. Considering their ecotoxic potential, Luckenbah and Epel [110] demonstrated that nitromusk and polycyclic musk compounds act as long-term inhibitors of cellular multixenobiotic resistance (MXR) defense systems mediated in aquatic mollusks by specific transport proteins.

4.2.2 Triclosan

Triclosan is essentially a pesticide (antibacterial agent), used in some healthcare facility soaps. It is also the most common antimicrobial agent in household liquid hand soap. It can be found in toothpaste, lip gloss, soap (solid and liquid), plastic products ranging from children's toys to cutting boards, and footwear [111]. It has been detected in human breast milk and serum samples from the general population [98, 112], and in the urine of 61% of 90 girls ages six to eight tested in a recent study spearheaded by Mount Sinai School of Medicine [73].

Triclosan kills microbes by disrupting protein production, binding to the active site of a critical carrier protein reductase essential for fatty acid synthesis, which is present in microbes but not humans. Available studies do not raise major concerns for human health, but some basic questions remain, including the safety of triclosan exposures in utero, and exposures in infancy through contaminated breast milk. Triclosan breaks down in the environment, including in tap water, to chlorinated chemicals that pose both environmental and health concerns [113].

Large quantities of triclosan are washed down drains and into wastewater treatment plants. A fraction is removed during water treatment, but the rest is discharged to lakes and rivers. Studies indicate that its interaction with sunlight results in the formation of methyl triclosan, a chemical that may bioacummulate in wildlife and humans [112, 114], as well as a form of

dioxin, which is a chemical linked to a broad range of toxicities including cancer [115]. The Canadian government limits the levels of dioxins and furans allowed as impurities in personal care products that contain triclosan. Triclosan was recently found in 58% of 139 US streams [116], the likely result of its presence in treated discharged wastewater. A safety standard for triclosan has not yet been set, and it does not require testing in tap water. However, it is believed that triclosan likely passes through standard water treatment processes to contaminate treated tap water supplies at low levels. New studies show that triclosan in tap water will readily react with residual chlorine from standard water disinfecting procedures to form a variety of chlorinated byproducts, including chloroform, a suspected human carcinogen [117].

Wildlife species are also contaminated with triclosan and its breakdown products; a recent European study found its breakdown product methyl triclosan in fish, especially concentrated in fatty tissue [113]. Triclosan is known to be acutely toxic to certain types of aquatic organisms, but little is known about its long-term effects on humans [118]. The chemical structure of triclosan is similar to that of diethylstilbestrol (DES), a non-steroidal estrogen, raising concerns about its potential to act as an endocrine disruptor. A recent study showed that triclosan can affect the thyroid gland, significantly altering frog metamorphosis at exposure levels equivalent to those currently found in the environment and human tissues, suggesting that triclosan may represent a potential health risk to human hormone action as well [119]. Studies have also found that triclosan has weakly androgenic effects but no estrogenic effects [120]. In addition, animal studies have shown that prolonged application of triclosan solution to the skin can cause dermal irritation in people with a specific sensitivity. There is no evidence that triclosan is a carcinogen or teratogen [121]. There is concern that the widespread use of antimicrobials such as triclosan in household products may promote antibiotic resistance in bacteria, although the current literature shows a possible association but no definitive link [122].

In addition to the PCPs mentioned above, some other categories like sunscreen agents, preservatives, and nutraceuticals recently got attention as possible emerging contaminants. As for now, however, the weight of evidence does not justify their treatment as immediate hazard to human or wildlife health.

4.3 Pharmaceuticals (Human Drugs and Veterinary Medicines)

Recent studies have also identified a number of pharmaceuticals as potential environmental contaminants that may adversely affect reproduction and development of biota in the environment [111, 123]. Some of these substances are not removed in traditional, or even advanced treatment systems, or under best management practices [124, 125]. Several of these substances have re-

cently been detected in well treated effluents and drinking water, showing that sewage treatment frequently does not affect the chemical structure, and, therefore, the toxicity of drugs [126–129]. Emerging data in Europe and North America suggests that these chemicals are widespread in the environment, especially in surface waters exposed to human or agriculture wastes [116, 130]. Consequently, pharmaceuticals often enter the environment at levels similar to better studied agrochemicals.

Traditionally, pharmaceuticals and personal care products have not been viewed as environmental pollutants [131]. However, the potential for these substances to cause a variety of physiological responses in non-target species has raised concerns for possible impacts on the environment. Although these substances are usually found at very low concentrations in the environment, continuous low-dose exposure to these complex mixtures, especially at sensitive life stages, may have significant effects on individuals, populations, or ecosystems. The ecological impact of long-term exposure to large mixtures of those essentially biologically active chemicals is also unknown. Many of these chemicals are known to be persistent in both treatment systems and in the environment. Chemicals found in sewage and manure, such as synthetic estrogens, are known to have biological consequences at extremely low exposures [132]. Exposure of biota to even low doses during critical or sensitive life-stages may have profound effects on development and reproduction for multiple generations.

Due to their intended use in human or veterinary medicine, pharmaceuticals are generally well studied and a large body of toxicological evidence directed to human health issues exists for most of them. Considering their ecotoxicity, however, the available evidence in most cases provides indications of acute effects in vivo for organisms at different trophic levels after short-term exposure, but extremely rarely after long-term chronic exposures. An excellent service called "The Pharmaceuticals in the Environment, Information for Assessing Risk" has been recently developed and is maintained at the National Centers for Coastal Ocean Science (NOAA), Center for Coastal Environmental Health and Biomolecular Research, USA [133]. The database provides information on prescribed amounts, levels detected in aquatic environments, chemical structure, molecular weight, octanol-water partition coefficients, water solubility, environmental persistence, general toxicity information, and specific toxicity levels of pharmaceuticals to five groups of organisms (algae, mollusks, finfish, crustaceans, and select terrestrial animals). Toxicity to terrestrial animals is provided as a general comparison to data found in toxicological literature. All of this information was obtained from available scientific literature and is provided to assist with indentification of locations where risks to aquatic organisms might occur.

Considering the ecotoxicity of human pharmaceuticals, most of the current knowledge is well summarized in several excellent review articles published during the last few years [111, 130, 134–136]. Summarizing the avail-

able data, it is clear that there is almost no data about bioaccumulation of pharmaceuticals in biota, and often there is no correlation between the acute toxicity and lipophilicity. Most of pharmaceuticals displayed their LC50 values above 100 mg/L, which classifies them as not being harmful to aquatic organisms. However, variability of data within the same, as well as between different species is considerable, often spanning one or two orders of magnitude. Nevertheless, the overall conclusion is that acute toxicity of pharmaceuticals may be only relevant in case of accidental spills. Chronic toxicity, however, appears to be more relevant to aquatic biota and numerous examples clearly point out that it cannot be derived from acute toxicity data by simple calculations.

Veterinary pharmaceuticals, on the other hand, were traditionally less covered in environmental and human health toxicity studies. Current livestock and aquaculture production practices include the use of a wide variety of pharmaceuticals to enhance animal health and efficient food production, including antimicrobials (antibiotics), growth enhancers, feed supplements, and other medicinal products. Recently, low levels of veterinary medicines were detected in soils, surface waters, and ground waters worldwide [137]. Although the environmental occurrence and associated impacts of some compounds, such as selected antibacterial compounds, have been investigated, the impacts of many other substances found in the environment are not well understood. As a result, questions have arisen about the effects of veterinary medicines on organisms in the environment and on human health.

The interest in veterinary pharmaceuticals as potential emerging contaminants has also stemmed from the proliferation of large-scale animal feeding operations (AFOs) during the last decade. The large number of animals produced creates a proportionately large volume of animal waste and associated emerging contaminants. In a reconnaissance study of liquid waste at swine AFOs in Iowa and North Carolina, US, multiple classes of antibiotics were detected ranging from ppb to ppm concentrations [138]. Compilation of data from liquid waste from swine operations between 1998 and 2002 found one or more antibiotics present in all of the samples. The data from these studies demonstrate that veterinary pharmaceuticals are excreted and frequently occur at detectable levels ranging from ppb to ppm concentrations in liquid and solid waste.

Research to document the presence of antibiotics in fish hatchery recently revealed the occurrence and persistence of antibiotics in medicated feed used in fish hatcheries [139]. It was discovered that ormetoprim and sulfadimethoxine persisted in water for longer periods of time than oxytetracycline in fish hatcheries. Oxytetracycline was detected more frequently in the samples of the intensive hatcheries than samples from the extensive hatcheries. Sulfadimethoxine concentrations were greater in the intensive hatcheries than the extensive hatcheries, but persisted up to 40 days after treatment in both types of fish hatcheries. In addition, antibiotics were de-

tected in untreated hatchery raceways, suggesting that recirculating water within a hatchery can lead to unintentional low-level exposure of antibiotics to healthy fish.

4.4 Nonculturable Biological Pathogens as Emerging Contaminants

Among the viruses infecting humans, many different types are excreted in high concentrations in the feces of patients with gastroenteritis or hepatitis and in lower concentrations in the feces or urine of patients with other viral diseases. Moreover, viruses are also present in healthy individuals, and, thus, high viral loads are detected in urban sewage and are regarded as environmental contaminants [140]. Some viruses, such as humanpolyomaviruses and some adenovirus strains, infect humans during childhood, thereby establishing persistent infections. In the case of many frequent adenoviral respiratory infections, viral particles may continue to be excreted in feces for months or even years afterward. There is available information about some waterborne pathogens, but the improvement in molecular technology for detecting viruses present in water has focused attention on new groups of viruses that could be considered emergent viruses in diverse geographical areas. Technical advances are then most readily associated with the concept of emergent microorganisms, which are defined as newly identified microorganisms, those already existent but characterized by a rapidly increasing incidence and/or geographical ambit, and those for which transmission through food or water has only recently been discovered. Several studies have confirmed that infectious diseases related to water are not only a primordial cause of mortality and morbidity worldwide but also that both the spectrum and incidence of many diseases related to water are increasing. Human polyomaviruses, hepatitis E virus (HEV), and human adenoviruses (HAdV) are three groups of viruses, which are being detected more often in the environment [141]. Adenoviruses, for example, are important human pathogens that are responsible for both enteric illnesses and respiratory and eye infections. Recently, these viruses have been found to be prevalent in rivers, coastal waters, swimming pool waters, and drinking water supplies worldwide. USEPA listed adenovirus as one of nine microorganisms on the Contamination Candidate List for drinking water, because their survival characteristic during water treatment is not yet fully understood. Adenoviruses have been found to be significantly more stable than fecal indicator bacteria and other enteric viruses during UV treatment, and adenovirus infection may be caused by consumption of contaminated water or inhalation of aerosolized droplets during water recreation.

In addition, many species of bacteria pathogenic to humans, such as *Legionella*, are thought to have evolved in association with amoebal hosts. Several novel unculturable bacteria related to *Legionella* have also been found in amoebae, a few of which have been thought to be causes of nosocomial

infections in humans [142]. A recent study done by Berk and colleagues in 2006 [143] revealed that it is over 16 times more likely to encounter infected amoebae in cooling towers than in natural environments. Several identified bacteria have novel rRNA sequences, and most strains were not culturable outside of amoebae. Such pathogens of amoebae may spread to the environment via aerosols from cooling towers. Therefore, studies of emerging infectious diseases should strongly consider cooling towers as a source of amoeba-associated pathogens.

Additional example is Campylobacter(s), which are emerging as one of the most significant causes of human infections worldwide, and the role that waterfowl and the aquatic environment have in the spread of disease is beginning to be elucidated [144]. On a world scale, Campylobacters are possibly the major cause of gastrointestinal infections. They are common commensals in the intestinal tract of many species of wild birds, including waterfowl. They are also widely distributed in aquatic environments where their origins may include waterfowl as well as sewage effluents and agricultural runoff. Campylobacters have marked seasonal trends and in temperate aquatic environments they peak during winter, whereas spring-summer is the peak period for human infection. Campylobacter species may survive, and remain potentially pathogenic, for long periods in aquatic environments. The utility of bacterial fecal indicators in predicting the presence of campylobacters in natural waters is questionable. Viable but nonculturable Campylobacter cells may occur, but whether they have any role in the generation of outbreaks of campylobacteriosis is unclear. The routine detection of *Campylobacter* spp. in avian feces and environmental waters largely relies on conventional culture methods, while the recognition of a particular species or strain is based on serotyping and increasingly on molecular methods.

4.5 Antibiotic Resistance Genes

Antibiotic resistance genes (ARGs) are another type of "biological" emerging environmental contaminants. Along with nanoparticles, they may be classical examples of indirect toxicants. The primary health concern in the case of ARGs is related to adverse outcomes of antibiotic's exposures resulting in selection for pathogen resistance or alteration of microbial community structures. The occurrence of ARGs was recently demonstrated in various environmental compartments including river sediments, irrigation ditches, dairy lagoons, and the effluents of wastewater recycling and drinking water treatment plants [145]. Some of ARGs were also present in treated drinking water and recycled wastewater, suggesting that these are potential pathways for the spread of ARGs to and from humans. On the basis of recent studies, there is a need for environmental scientists and engineers to help address the issue of the spread of ARGs in the environment.

4.6 Nanomaterials

I close this section with nanomaterials – the concerns of the future and "real" emerging contaminants. Engineered nanomaterials are commonly defined as materials designed and produced to have structural features with at least one dimension of 100 nanometers or less. Such materials typically possess nanostructure-dependent properties (e.g., chemical, mechanical, electrical, optical, magnetic, biological), which make them desirable for commercial or medical applications. However, these same properties potentially may lead to nanostructure-dependent biological activity that differs from and is not directly predicted by the bulk properties of the constituent chemicals and compounds.

The potential for human and ecological toxicity associated with nanomaterials and ultrafine particles is a growing area of investigation as more nanomaterials and products are developed and brought into commercial use. To date, few nanotoxicology studies have addressed the effects of nanomaterials in a variety of organisms and environments. However, the existing research raises some concerns about the safety of nanomaterials and has led to increased interest in studying the toxicity of nanomaterials for use in risk assessment and protection of human health and the environment. A new field of nanotoxicology has been developed to investigate the possibility of harmful effects due to exposure to nanomaterials [146]. Nanotoxicology also encompasses the proper characterization of nanomaterials used in toxicity studies. Characterization has been important in differentiating between naturally occurring forms of nanomaterials, nano-scale byproducts of natural or chemical processes, and manufactured (engineered) nanomaterials. Because of the wide differences in properties among nanomaterials, each of these types of nanoparticles can elicit its own unique biological or ecological responses. As a result, different types of nanomaterials must be categorized, characterized, and studied separately, although certain concepts of nanotoxicology, primarily based on the small size, likely apply to all nanomaterials.

As materials reach the nanoscale, they often no longer display the same reactivity as the bulk compound. For example, even a traditionally inert bulk compound, such as gold, may elicit a biological response when it is introduced as a nanomaterial [147]. The earliest studies investigating the toxicity of nanoparticles focused on atmospheric exposure of humans and environmentally relevant species to heterogeneous mixtures of environmentally produced ultrafine particulate matter (having a diameter < 100 nm). These studies examined pulmonary toxicity associated with particulate matter deposition in the respiratory tract of target organisms [148–151]. Epidemiological assessments of the effects of urban air pollution exposure focusing on particulate matter produced as a byproduct of combustion events, such as automobile exhaust and other sources of urban air pollution, showed a link in

test populations between morbidity and mortality and the amount of particulate matter [152, 153].

Laboratory-based studies have investigated the effects of a large range of ultrafine materials through in vivo exposures using various animal models as well as cell-culture-based in vitro experiments. To date, animal studies routinely show an increase in pulmonary inflammation, oxidative stress, and distal organ involvement upon respiratory exposure to inhaled or implanted ultrafine particulate matter. Tissue and cell culture analyses have also supported the physiological response seen in whole animal models and yielded data pointing to an increased incidence of oxidative stress, inflammatory cytokine production, and apoptosis in response to exposure to ultrafine particles [154–157]. These studies have also yielded information on gene expression and cell signaling pathways that are activated in response to exposure to a variety of ultrafine particle species ranging from carbon-based combustion products to transition metals. Polytetrafluoroethylene fumes in indoor air pollution are nano-sized highly toxic particles [158]. They elicit a severe inflammatory response at low inhaled particle mass concentrations, suggestive of an oxidative injury.

In contrast to the heterogeneous ultrafine materials produced incidentally by combustion or friction, manufactured nanomaterials can be synthesized in highly homogenous forms of desired sizes and shapes (e.g., spheres, fibers, tubes, rings, planes). Limited research on manufactured nanomaterials has investigated the interrelationship between the size, shape, and dose of a material and its biological effects, and whether a unique toxicological profile may be observed for these different properties within biological models. Typically, the biological activity of particles increases as the particle size decreases. Smaller particles occupy less volume, resulting in a larger number of particles with a greater surface area per unit mass and increased potential for biological interaction [159]. Recent studies have begun to categorize the biological response elicited by various nanomaterials both in the ecosystem and in mammalian systems. Although most current research has focused on the effect of nanomaterials in mammalian systems, some recent studies have shown the potential of nanomaterials to elicit a phytotoxic response in the ecosystem. In the case of alumina nanoparticles, one of the US market leaders for nano-sized materials, 99.6% pure nanoparticles with an average particle size of 13 nm were shown to cause root growth inhibition in five plant species [160].

Charge properties and the ability of carbon nanoparticles to affect the integrity of the blood-brain barrier as well as exhibit chemical effects within the brain have also been studied. Nanoparticles can overcome this physical and electrostatic barrier to the brain. In addition, high concentrations of anionic nanoparticles and cationic nanoparticles are capable of disrupting the integrity of the blood-brain barrier. The brain uptake rates of anionic nanoparticles at lower concentrations were greater than those of neutral or cationic formulations at the same concentrations. This work suggests that

neutral nanoparticles and low concentration anionic nanoparticles can serve as carrier molecules providing chemicals direct access to the brain and that cationic nanoparticles have an immediate toxic effect at the blood-brain barrier [161, 162].

Tests with uncoated, water soluble, colloidal C60 fullerenes have shown that redox-active lipophilic carbon nanoparticles are capable of producing oxidative damage in the brains of aquatic species [161]. The bactericidal potential of C60 fullerenes was also observed in these experiments. This property of fullerenes has possible ecological ramifications and is being explored as a potential source of new antimicrobial agents [163]. Oxidative stress as a common mechanism for cell damage induced by nanoparticles and ultrafine particles is well documented; fullerenes are model compounds for producing superoxide. A wide range of nanomaterial species have been shown to create reactive oxygen species both in vivo and in vitro. Species which have been shown to induce free radical damage include the C60 fullerenes, quantum dots, and carbon nanotubes. Nanoparticles of various sizes and chemical compositions are able to preferentially localize in mitochondria where they induce major structural damage and can contribute to oxidative stress [164].

Quantum dots (QDs) such as CdSe QDs have been introduced as new fluorophores for use in bioimaging. When conjugated with antibodies, they are used for immunostaining due to their bright, photostable fluorescence. To date, there is not sufficient analysis of the toxicity of quantum dots in the literature, but some current studies point to issues of concern when these nanomaterials are introduced into biological systems. Recently published research indicates that there is a range of concentrations where quantum dots used in bioimaging have the potential to decrease cell viability, or even cause cell death, thus suggesting that further toxicological evaluation is urgently needed [165, 166]. However, the research also highlights the need to further explore the long-term stability of the coatings used, both in vivo and exposed to environmental conditions.

5 Discussion

5.1 Regulatory Perspective and Public Concerns

In 2004, the environmental campaign group World Wide Fund (WWF) tested the blood of government ministers from 13 EU Member States for chemicals that can negatively affect human health and wildlife. WWF found on average 37 out of the 103 tested substances in the ministers' blood [167]. Further, it is clear that the EU citizens are concerned. In a recent survey, the impact of chemicals used in everyday products came fifth in a list of 15 environ-

mental issues of concern. When asked about which issue they feel they lack information, citizens cited chemicals first [168]. Do they have reason(s) to be concerned? Undoubtedly, the answer is positive – the overview of the "chemical world", which is in this chapter concentrated only to today's man-made emerging contaminants, clearly suggests that there are real human and environmental health problems that have to be addressed. Considering the issue of chemical contamination, all critical parties – regulators, risk managers, industry sector, politicians, and, finally, scientists – do not offer answers and solutions needed for citizens to be less concerned.

Contamination of water supplies is an evolving problem and will remain an issue as long as technological change continues. Some of the contaminants now being targeted by researchers may come out with a clean slate, while others may require additional scrutiny. One of the hopes of today's researchers is that more sophisticated science will help speed the process of identifying and remedying the problems, before damage to either human health or the environment occurs. In any case, science and regulation must continue to evolve and change, as it has been the case in the past few years, to respond to new needs presented by chemicals and our increasing knowledge of them. At present, however, regulatory communities are placed in a reactive, rather than proactive, position with respect to identifying contaminants and addressing public concern. The current lists of environmental pollutants evolved from those established in 1970s and are mainly focused on conventional "priority pollutants" often referred to as "persistent organic pollutants" (POPs). As was elaborated, these chemicals represent only a tiny part of potential pollutants [1, 2] and biological systems may obviously suffer exposure to many more chemicals stressors, only a small number of which is regulated. Therefore, only a small proportion of potentially hazardous chemicals is toxicologically evaluated, and even smaller number of them is officially regulated.

This position is further emphasized in situations where federal funding is provided only on a short-term basis and only for specifically identified research needs, which by definition are reactionary calls to fill data gaps. Although this approach generates short-term products for stakeholders, it often leads to fragmentary, low profile science. In the long term, such goal-oriented approach to environmental funding does not allow for exploratory research that can be used to anticipate future environmental issues. Unfortunately, in the US, for example, there is no competitive funding scheme for the discovery of new contaminants. In addition, no cohesive plan exists to proactively screen and identify all contaminants of potential concern. On the other hand, both Canada and the EU are actively developing plans that will place them in positions from which they can anticipate future environmental issues. The Registration, Evaluation, and Authorization of Chemicals (REACH) regulation in the EU is a good example [169]. Entered into force in June 2007, it requires that manufacturers of substances and formulators register and provide prescribed (eco)toxicological data for all substances with

a volume >1 metric ton per year. In contrast, the USEPA has taken a different tack by sponsoring a voluntary program called the High Production Volume (HPV) Challenge Program [170]. Since the program's inception in 1998, >2200 chemicals have been "adopted" by chemical manufacturers and importers. Unfortunately, this number is small in comparison with the number of chemicals included under REACH, and >200 HPV chemicals are still without the promise of toxicity testing.

5.2 (Eco)toxicological Constraints

As may be realized from this overview, (eco)toxicologists often seems to know too little too late, and are far too slow to respond to numerous chemicals that enter the market every day. Moreover, most of (eco)toxicological testing is done using traditional acute toxicity test protocols. As was reliably demonstrated with pharmaceuticals, acute toxicity cannot always serve as a reliable proxy for chronic toxicity effects encountered in real environmental situations. Certain substances may elicit adverse effects weeks, months or years after exposure. Carcinogenicity is a classical example – an ultimate adverse outcome difficult to characterize regarding causal connections. Consequently, chronic exposure assessments cannot be avoided and proper toxicological characterization will probably continue to be a time-consuming process.

The array of chemicals in use will likely continue to diversify and grow with changing use patterns in human populations and animal production facilities. Rapid developments in the pharmaceutical industry will also continue to quickly add to the vast number of chemicals already entering the environment. Due to the ever-increasing potency and specificity of pharmaceuticals, new substances may be of even greater concern for the environment. New approaches for testing and new ways of thinking about new materials are also necessary. The diverse routes of exposure, including inhalation, dermal uptake, ingestion, and injection, can present unique toxicological outcomes that vary with the physicochemical properties of the nanoparticles in question.

The likelihood of constantly introducing new chemicals to commerce pose inevitable doubts as to whether the chemical-by-chemical approach to toxicological testing and regulation of water pollutants will continue to be sustainable. In the past, studies have focused on the effects of single chemicals because chemicals are usually regulated singly. However, chemicals are always present as complex mixtures, thus some might say the regulation approach is naïve. Thus scientists are increasingly focusing on the toxicity of mixtures of chemicals, acknowledging that the toxicity expressed may be a result of additive or multiplicative effects, depending on interactions with other chemicals present in the environment. Furthermore, the issue becomes even more complex taking into account potential toxicity of numerous metabolites being generated from parent compounds.

An alternative approach, formalized as "toxicity apportionment" has been recently proposed [2]. The main principle of this approach would be to assign toxicity according to the total numbers of stressors present, without the need to know their identities in advance. The apportionment approach is especially valuable in accounting for all toxicants sharing the same mechanism of action. As was proposed, water monitoring programs based on that framework should utilize biomarkers and biotests designed around evolutionary biochemical features and mechanisms of action rather than individual chemical entities. This approach may indeed be the best way to simultaneously account for multitude of contaminants having the same mechanism of action, chemicals newly introduced to the market, and pollutants of the future. Looking from the cost-benefit side and trying to obtain relevant toxicological answers in a short time, an efficient screening protocol similar to that shown in Fig. 1, may be based on the extensive use of a series of small scale and in vitro biotests, used to rapidly and sensitively screen for the presence of contaminants of concern, including emerging contaminants, addressing both acute and chronic toxicity and utilizing test species on different levels of biological organizations. It can be used for testing of single chemicals and complex environmental samples. Such a battery of mechanism-based bioassays could be easily incorporated into monitoring efforts.

Nevertheless, whilst they are able to indicate the presence of certain groups of substances in well understood media based on a toxic response, caution is needed in broadening the application of in vitro tests to complex media such as effluents. In vitro tests that typically utilize genetically modified cells, yeast, or bacterial strains, demonstrate promising advantages such as speed, low cost and the ability to give an indication of specific toxicity that usually is not expressed in acute toxicity tests. However, they have to date only been used to a limited extent on effluents, making interpretation of test results difficult or in some cases impossible. Additional experience will be essential to improve the interpretation of test results and their relationship to actual environmental impacts. At present, even the best validated in vitro bioassays are only suitable as an initial screening step to prioritize effluents or effluent fractions for further study. In vivo tests with carefully selected indicator species are more appropriate to assess direct toxicity and should preferably be used for risk assessment purposes. Furthermore, bioassays can give both false negative and false positive results. False negative results may fail to highlight real health or environmental risks; false positives may imply health or environmental risks where, in fact, there are none. Due to the high sensitivity of these tests, false positives are likely when applied to complex mixtures like effluents.

Therefore, methods are now available that detect tiny quantities of chemicals which may potentially be hazardous. However, questions remain about which chemicals are responsible when positive results are obtained from drinking water, wastewater, freshwater and seawater, soil, mud, or any other sample. For effluents, it is a challenge that samples generally contain many compounds,

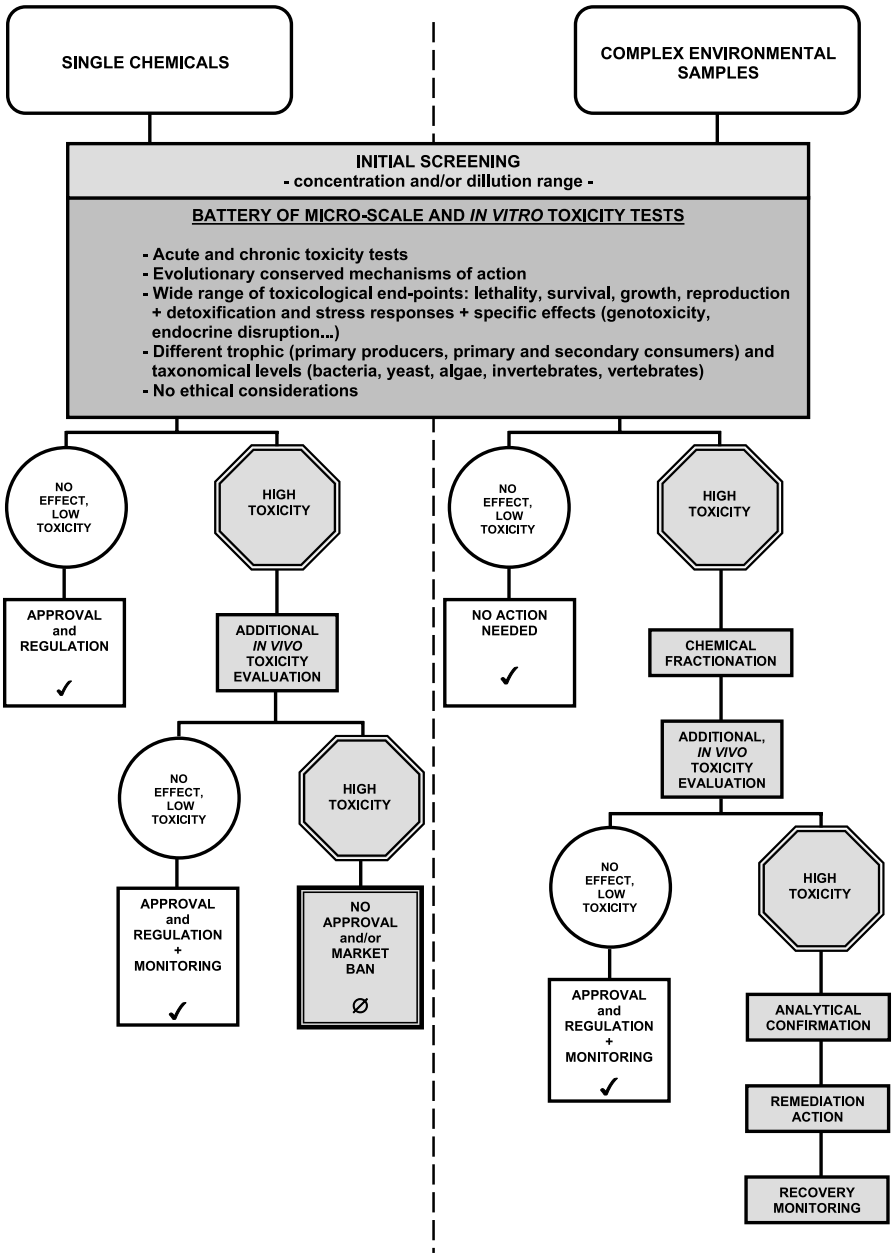

Fig. 1 Flow chart presentation of the possible (eco)toxicological protocol for rapid screening and characterization of single chemicals and complex environmental samples

resulting in false positives being frequently obtained. In the case of a positive response, the sample may be split up and analytical methods used to try and identify the responsive chemicals. Since these tests are highly sensitive and specific to the cell type used, the relevance of positive results for other species, living animals and longer-term exposures is the subject of ongoing studies. Consequently, a positive assay result should always be complemented with an in vivo assay and analytical detection to confirm the response. Only additional studies – coupled with a proper risk analysis, taking exposure into account – can confirm if the response indicates a genuine environmental risk.

Finally, regardless of the obstacles described, the most important concern regarding the exposure of aquatic and terrestrial organisms to emerging contaminants may be our inability to detect subtle health effects – imperceptible changes ranging from modification or reversal of attraction, behavioral changes related to feeding, matting, predator avoidance, or directional sensing. The changes we may see on the surface would simply be attributed to natural adaptation or any other form of natural changes. This concept of subtle changes, formalized at first by Kurelec in 1993 [171] as the genotoxic disease syndrome (GDS) was described as gradual accumulation of a wide spectrum of toxic events, none of which alone results in an easily detected adverse outcome. However, the final outcome would be an ultimate and often irreversible biological damage – species loss and decrease in biodiversity, unexpected and unexplained due to our inability to detect and act timely. These subtle, cumulative effects could make current toxicity-directed screening strategies largely useless in any effort to test waste effluents for toxic end points. At the moment, unfortunately, in the field of environmental toxicology there is no sound scientific answer to this critical issue. The raise of -omics techniques, however, especially genomics approach based on high-density microarray methodology, may be a future solution theoretically capable of detecting even subtle changes in gene expression patterns.

6 Conclusions and Future Directions

In this article, we briefly summarized major human end environmental health effects related to the most prominent categories of emerging contaminants, along with critical (eco)toxicological drawbacks and prerequisites needed for environmentally accountable risk characterization. The most important messages from this chapter, those we want for any reader to take into consideration are:

1. The threat posed by numerous emerging contaminants present in industrial and municipal waste is serious, poorly characterized, and should not be underestimated;

2. The research capacity of (eco)toxicology is at the moment far beyond capacity of analytical chemistry to detect new, emerging contaminants, and even more distant from the capacity of industry sector to design and introduce new chemical entities, likely "emerging" contaminants of the future;
3. Chronic, low-level exposure assessments do not have any scientifically sound alternative and should represent obligatory part of (eco)toxicity characterization of single chemicals and complex environmental mixtures;
4. The necessary improvements in the field of (eco)toxicology will not be possible without major shift in the regulatory arena, including significant changes in the environmental funding schemes.

Countries that adopt proactive approaches, such as the EU REACH initiative, will be afforded distinct environmental, economic, and scientific advantages, because they will be better serving human and nonhuman populations and ecosystems, with tangible savings to the healthcare and environment protection costs. Without the adoption of proactive plans to identify contaminants before they emerge, regulatory communities that remain in reactionary modes will be unable to fully serve the needs of the populations they represent.

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