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Pollutants of Emerging Concern in Ecotoxicology and Environmental Chemistry Studies.

Ecotoxicological Research and Ecological Risk Assessment

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Abstract. The scientific fields of ecotoxicology and environmental chemistry are advancing rapidly covering the study of emerging pollutants in the last decade. This review aims to present recent ecotoxicological research projects in emerging areas of environmental pollution. Among other developments the use of marine organisms as bioindicators of chemical water pollution is gaining great prominence in environmental studies. Ecotoxicological studies play an important role in measurements of environmental pollution by heavy and trace metals, polycyclic aromatic hydrocarbons (PAHs), fertilizers, halogenated organic compounds (chlorinate, brominated and fluorinated), pesticides, flame retardants, pharmaceuticala and metabolites, nanoparticles from novel materials, municipal and industrial waste mixtures, disinfectants, wood preservatives, flame retardants, plasticizers, and a great variety of other toxic compounds. In addition, ecotoxicological studies investigate the molecular, biochemical and cellular underlining mechanisms and effects on the environment, its biota and ecosystem biodiversity. Also, ecotoxicology and environmental chemistry investigate the transfer of chemical pollutants along terrestrial food chains and marine food webs, the biomagnification of toxic substances and the possible risks. These studies are used to produce ecological risk assessment models that can be useful to evaluate levels of pollution and integrate a great variety of factors for future developments. Ecological risk assessments support management decisions for positive actions to reduce pollution and future monitoring of areas under threat. The development of the conceptual models of eccological risk assessment can be paralleled by the continuous upgrade of specific environmental measurements and dedicated software. Areas of great interest for ecotoxicological studies are mainly naval ports, sea dredging activities in coastal areas, polluted sediments in marine areas, surface waters with eutrophication problems (nitrate and phospheate pollution), marine areas with high concentration of heavy metals and polluted marine food webs. Also, environmental pollution investigations can be applied in polluted sites by municipal effluents and industrial solid and liquid waste. Ecotoxicological studies are needed for monitoring of off-shore installations (petroleum and gas extraction platforms or terminals), industrial sites and agricultural activities in cases of accidents and extensive marine pollution disasters.

Introduction: Ecotoxicological studies

The scientific determination of ecotoxicology is very recent. In 1969 the French toxicologist René Truhaut coined the term ecotoxicology as "*the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal, vegetable and microbial, in an integral context".*[1] In the last decades toxicologists and environmental scientists with numerous publications projected the new dimensions of ecotoxicological studies. They emphasized that ecotoxicology differs from environmental toxicology in that it integrates the effects of pollutants (or contaminants or stressors) across all levels of biological organisation from the molecular to whole communities and ecosystems, whereas environmental toxicology focuses upon adverse effects at the level of the individual subject (animal, plant, humans) and below.[2-5]

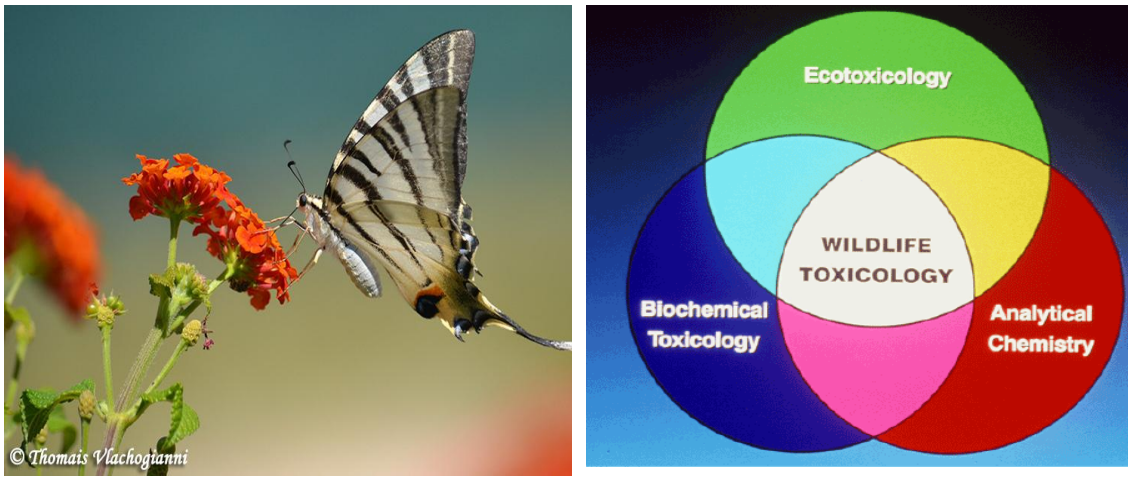


Figure 1. Environmental toxicology and ecotoxicology study the effects of pollutants or contaminats, their transformations, and biochemical toxic mechanisms in animals, plants and microorganisms and their adverse toxicological effects on ecosystems.

In the past environmental pollutants which caused extensive pollution to the water resources, land and air were categorised as to their extent, toxic mechanisms, biomagnification and adverse effects on the enironment and ecosystems. In the last decades new technological advances produced new and persistent pollutants with high capacity to be absorbed by plants and animals and to be distributed among food webs. Examples are trace elements, perfluorinated compounds, gasoline additives (e.g. MTBE), plasticizers, chemical with endoctine disrupter properties, pesticides, manufactured nanomaterials and nanoplastics, disifection byproducts,

pharmaceutical metabolic compounds, veterinary pharmaceuticals and antibiotics, sunscreens and ultravioled filters, etc., which were characterised for their bioaccumulation to marine organsims and adverse effects on ecosystems.[6-9]

New trends in ecotoxicological investigations

In the last decades the fields of environmental chemistry, toxicology and ecotoxicology have establish multiple missions to investigate the global implications of toxic chemical substances towards environmental pollution, effects on ecosystems and human health. Developed countries with large industrial chemistry installations and commercial involvement in the distribution of chemicals have advanced new policies and actions on the protection of the environment and human health. These include the European Union's chemicals policy Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH; http://ec.europa.eu/enterprise/reach/reach/indexen.htm), the Strategic Approach to International Chemicals Management (SAICM), and the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; http://ec.europa.eu/enterprise/reach/ghs/indexen.htm), which could replace risk assessment by hazard assessment with little or no consideration of exposures or probability of adverse effects in the environment.

Ecotoxicological biomarkers can provide information for toxicity status and answers for the development of control strategies and precautionary measures in respect to the European Water Framework Directive-Intergrative River Basin Management. In the USA the organization Environmental Protection Agency (EPA) has advanced numerous ecotoxicological investigations of environmental pollution. It is recognised that further challenges of environmental risk assessment are connected with emerging environmental contaminats (pollutants) and are recently comprehensively investigated by U.S. EPA-Science Advisory Board.[10,11]

The U.S. EPA is currently considering revision of quatic life criteria for contaminants of emerging concern under the Clean Water Act. The U.S. EPA working group recognizes that some contaminants of emerging concern (CECs) are likely never to reach concentrations in the environment that may induce acute lethality and toxicity, except for scenarios of accidental releases or as mixtures. There is

concern that chronic effects may occur at environmentally realistic concentrations. EPA's Office of Pesticide Programs (OPP) and the Office of Water (OW) assess the effects of pesticides on aquatic ecosystems using approaches that afford a high degree of protection for aquatic life. Also, EPA scientists are considering the evaluation of the potential impact of contaminants of emerging concern (CECs), including pharmaceuticals and personal care products (P&PCPs), which are increasingly being detected at low concentrations in surface water, on aquatic life.[12]

The ECETOC (**European Centre for Ecotoxicology and Toxicology of Chemicals**, Brussels) is a scientific agency promoting ecotoxicological research and scientific reviews on emerging toxic and hazardous chemicals. It and is an independent, non-profit, non-commercial and non-governmental organisation which provides a scientific forum through which the extensive specialist expertise of manufacturers and users of chemicals could be harnessed to research, review, assess and publish studies on the ecotoxicology and toxicology of chemicals.[13]

Figure 2. Ecotoxicological research institutes and toxicology laboratories in universities are cooperating in environmental research and promotion of comprehensive ecotoxicological risk assessment of new chemicals and emerging toxic substances of new consumer products and chemicals in municipal effluents.

In 2001 European Centres of Ecotoxicology and Environmental Toxicology created a network. It was called **PEER, Partnership for European Environmental Research**. The PEER network is a partnership of 7 large European environmental research centres: Alterra (The Netherlands), CEH (Centre for Ecology & Hydrology,

United Kingdom), Cemagref (Environmental Sciences and Technologies Research Institute, France), JRC/IES (European Commission, Joint Research Centre, Institute for Ecology and Sustainability, Italy), NERI (National Environmental Research Institute, Denmark), SYKE (Finnish Environment Institute, Finland), UFZ (Helmholtz Centre for Environmental Research, Germany). The PEER network is a European research leader in integrating knowledge and expertise for sustainable development, in support of policy makers, industry and society.[14]

PEER network scientists aim for an improved biologically relevant exposure assessment. They promote comprehensive effect assessment at several biological levels. Their studies focus on biological traits that can be used for Environmental Risk Assessment (ERA) as promising tools to better understand relationships between structure and functioning of ecosystems. The use of modern high throughput methods could also enhance the amount of data for a better risk assessment. Improved models coping with multiple stressors or biological levels are necessary to answer for a more scientifically based risk assessment. Those methods must be embedded within life cycle analysis or economical models for efficient regulations. Joint research programmes involving humanities with ecological sciences should be developed for a sound risk management.[14]

The Scandinavian countries (or Nordic countries) had traditionally a large number of centres and agencies dealing with ecotoxicology problems. Many environmental and toxicology departmens in universities and state organizations have initiated new investigations in environmental pollution and threats to ecosystems by toixc substances. A list of ecotoxicological expertise in Denmark, Finlad, Norway and Sweden was published in 1991 by the Nordic Council of Ministers (Copenhagen). New facilities for toxicology studies are established in Scandinavian countries (e.g. Swetox, Sweden's new toxicology centre collaborating with 11 of Sweden's leading universities from 2014].[15-17]

The European Commission established in 2004 the **Scientific Committee on Emerging and Newly Identified Health Risks** (SCENIHR) to provide the Commission with unambiguous scientific advice on the safety of a series of issues requiring a comprehensive assessment of the risks, such as new technologies, medical devices, etc. The Scientific Committee is composed of a maximum of 13 members on the basis of their skills and scientific experience. If there are specific scientific or

technical quiestions the committee may enlist the support of up to 6 associated members on the basis of their expertise. Also, the committee may turn to specialised external scientific experts for advice. The SCENIHR complies with the prinicples of independence, transparency and confidentiality. The aim is to resolve environmental and health risks on emerging pollutants. It provides opinions on emerging or newly-identified health and environmental risks and on broad, complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies. In 2016, it was succeeded by the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER). Some of the potential areas of activity include: antimicrobial resistance, environmental threats by new technologies (e.g. nanotechnologies, endoctine disrupters, persistent chemicals, new pesticides), new medical devices, physical hazards that cause advarse health effects (e.g. noise, electromagnetic fields, ozone depletion), tissue engineering techniques, blood products, environmental causes of fertility reduction, cancer causing substances, interaction of risk factors (synergic effects, cumulative effects), methodologies for assessing new environmental risks.[18,19]

In the last decade there are numerous ecotoxicological laboratories in universities undertaking ecotoxicological studies on various areas of emerging toxicological issues. For example the laboratory of Prof. Francesco Regoli, Dipartimento di Scienze della Vita e dell'Ambiente, Polytechnic University of Marche, Ancona, Italy. Prof. Regoli is Chair of "Ecotoxicology and Biological and Ecological Risk Assessment". His research activity is mostly focused on the use of marine organisms as bioindicators of chemical pollution and environmental disturbance, with particular emphasis to ecotoxicological effects, emerging pollutants, trophic transfer of chemicals, oil and chemical spills, vulnerability of polar areas, impact of dredging and off-shore activities, algal toxins, promotion of models of ecological risk assessment. [details in: http://www.disva.univpm.it/content/ecotoxicology-and-environmental/chemistry?language=en].

Another interesting Ecotoxicology Laboratory is in the University of Portsmouth, England. The ecotoxicology and environmental monitoring group works

as collaboration between the School of Biological Sciences, School of Earth and Environmental Sciences and the School of Pharmacy and Biomedical Sciences. The laboratory focus on pure and applied aspects of biological and environmental sciences associated with how humans impact their environment. Staff members within this group use a wide variety of model organisms and techniques within the field and laboratory to study toxicology, aquatic, terrestrial and air pollution monitoring and the impact of climate change. Current funding includes NERC, BBSRC, NC3Rs, Environment Agency, European Union and many industry sponsors.[20]

There is also a network, called NORMAN, of reference laboratories in European countries and research centres for monitoring emergency environmental substances. The NORMAN network (www.norman-network.net), aims for the investigation at least 700 substances (emerging pollutants, EPs), their metabolites and transformation products, listed and categorized into 20 classes. These toxic compounds have been identified in the European aquatic environment and because of their toxicity and adverse environmental and health effects need to be studied in the near future and to establish their ecotoxicological signifinance. The NORMAN network (started in 2005 with the financial support of the European Commission) focus on the enhancement and exchange of information among ecotoxicological laboratories on emerging environmental pollutants and encourages the validation and harmonisation of common masurements methods and monitoring tools Seeks also to promote synergies between research teams from different countries.[21]

Emerging pollutants, their metabolites and transformation products

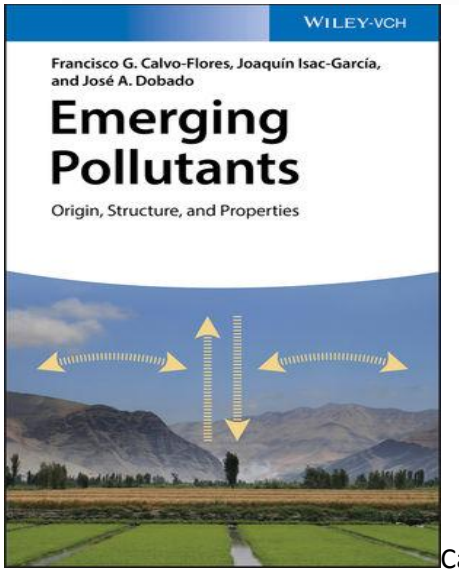
The majority of Emerging Pollutants (EPs) are synthetic or naturally occurring chemicals or mixtures (at very low concentrations) that are not at present commonly monitored in the environment, but which have the potential to enter the environment, bioaccumulate and cause adverse ecological effects. EPs are currently not included in routine monitoring programmes and their fate, behaviour and ecotoxicological behaviour is not known. The numbers of ecotoxicology studies worldwide have increased in the past decade, resulting in the discovery of several

new environmental threats, toxicological mechanisms, trends of biomagnification and establishing new methodologies in modeling of risk assessment. The EPs are preferably termed as “contaminants of emerging concern”. Environmental scientists from many decades in the past were dealing with the issues of new pollutants. For example lead (Pb) pollution of air and water was a big problems in the 1950s, Arsenic (As) toxicity and increased skin cancer epidemic in agricultural workes was another toxicological issue, asbestos fibers and its association with lung cancer was well known in the 1960s, environmental pollution by persistent polychlorinated compounds like DDT and other pesticides was investigated in the 1970s. In the last decades new and emerging pollutants (or contaminants), such as transformation or metabolic products of pharmaceuticals, cyanotoxins, personal care products, nanoparticles, flame retardants, became research targets of toxicological and ecotoxicological studies.[22]

The majority EPs can be released from point pollution sources, e.g. waste water treatment plants from urban or industrial areas, or from diffuse sources through atmospheric deposition or from crop and animal production. EPs are categorized into more than 20 classes related to their origin.The prominent classes of emerging pollutans or contaminants are: [22-24]

a. **Pharmaceuticals** and **veterinary medicines** (urban, stock farming),
b. **Pesticides and other synthetic products** (agriculture, gardens),
c. **Neonicotinoids pesticides**. Very popular with farmers, a new class of pesticides produced in significant quantities (imidacloprid, acetamiprid, thiamethoxam, etc.) act specifically to the nicotinic acetylcholine receptor (nAChR) of insects. Recently, major problem toxicity on pollinator honey bees. Regulatory agencies face a difficult issue for banning or restricting their use.
d. **Roundup (Glyphosate).** Pesticide with widespead use
e. **disinfection by-products** (urban, industry),
f. **wood preservation** and industrial chemicals for preservations of materials (industry),
g. **flame retardants**. Some f.r. can cause environmental pollution and health riks after exposure. In the past some f.r. have been banned for use.
h. **Plasticizers and their metabolites** are measured in sewage treatment plant effluents. Plasticizers, are additives used to increase the flexibility or plasticity, such as bisphenol A or phthalates and are particularly recognized as endocrine disruptors which have been banned or strictly regulated.

i. **Various fluorinated compounds,** mainly perfluoroalkyls and polyfluoroalkyl (PPFAs) have made it to the market and have since has been targeted for stricter regulations given their environmental properties.
j. **cyanotoxins, cyanobacteria** are among the first biological organisms on Earth, so not really a newcomer. Eutrophication of water bodies and global warming are contributing to algal blooms. New and improved analytical techniques now allow scienyists to better detect cyanotoxins produced by those organisms.
k. **New cosmetics**, **synthetic musks, fragrances**. A wide range of washing and cleaning agents and personal care products with toxicological issues appeared in the last decades.
l. **Nanoparticle, nanomaterials** (less than 100 nanometers), new products with quite challenging toxciological profile. The risk assessment of nanomaterials needs to be re-evaluated. Carbon-based nanoparticles such as carbon nanotubes or fullerenes and metal-based nanoparticles such as metal oxides or quantum dots.
m. **Semivolatile** organic compounds as contaminants
n. **Suspended particulate matter** excerting toxicological inffluence
o. **Hydroxylatedf polybrominated diphenyl ethers** with toxic effects



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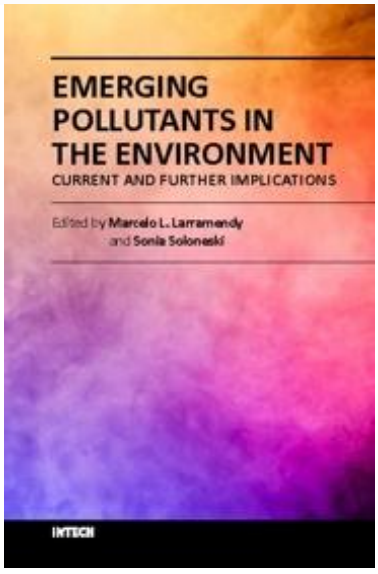

Larramendy ML, Soloneski S (Eds), *Emerging Pollutants in the Environment. Current and Further Developments*. InTech, London, 2015.

Figure 3. Emerging pollutants have become the subject of many publications in recent years and their potential to cause widespread problems in the environment.

Ecotoxicological problems with pharmaceuticals and their metabolites

Although pharmaceuticals, their metabolites and breakdown products have been present in water resources (wastewater treatment plants, rivers, lakes, surface sea water, etc) for decades, their concentrations in the environment have only

recently begun to be quantified. Also, in the last decade toxic pharmaceutical contaminants were recognised as potentially hazardous to ecosystems. The development of new analytical techniques, such as liquid chromatography coupled to mass spectrometry [LC-MS], tandem MS [MS^2], or LC-MS^2) has allowed the detection of extremely low concentrations of pharmaceuticals and other emerging pollutants, at levels of μg or ng/L or even pg/L (pico grams = 10^{-12} g).[25-27]

Over the last 20 years, pharmaceuticals have been receiving increasing attention as potential bioactive chemicals in the environment. Pharmaceuticals are considered as emerging pollutants in waterbodies because they still remain unregulated or are currently undergoing a regularization process. New technology and carbon filters in watewater treatment plants remove substantial amounts of these contaminants. Although pharmaceuticals are prevalent at small concentrations, their presence can affect water quality (rivers, lakes, surface water), drinking water supplies, and ulitmately cause adverse impact on sensitive ecosystems and human health.[28-30]

There are many ecotoxicological studies of pharmaceutical pollutants on acute effects in organisms belonging to different trophic levels (i.e. algae, zooplankton and other invertebrates and fish) but chronic toxicity studies are very limited. Acute toxicity data is only valuable when accidental discharge of the drugs occurs. Normally, the environmental concentrations of pharmaceuticals are very low to cause detectable adverse effects. Bioaccumulation and chronic toxicity tests are scarce probably due to the complex experimental work involved. However, recent development of sensitive methods for identification and quantification of drugs enabled to devise their distribution patterns in several environmental samples, thus highlighting the more relevant therapeutic classes in terms of environmental contamination.[31-33]

The most relevant pharmaceutical classes in terms of environmental contamination have been selected by collecting research data from ecotoxicity tests and other studies. One review set out the most appropriate active drug substances used in ecotoxicity tests from 134 articles published in the period 1997-2009. These drugs were: antibiotics, blood lipid lowering agents, sex hormones, ano-steroidal

anti-inflammatory drugs, antiepileptics, beta-blockers, antidepressants, antihypertensives, X-ray contact media, antiacids, antineoplastic drugs, β2 – sympathomimetics, oral antidiabetics, antipsychotics, etc.[31]

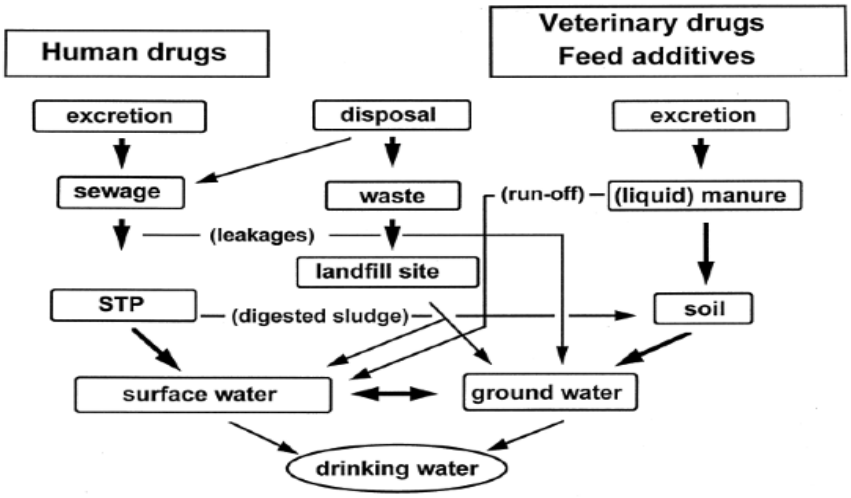

Figure 4. Pharmaceuticals for human therapeutic treatments and veterinary drugs have been identified in municipal and agricultural waste effluents. As emerging pollutants pharmaceuticals are of environmental concern and their adverse effects were studied by numerous ecotoxicological studies in the last decade.

Antibiotics as emerging toxic pollutants. According to various studies antibiotics are considered as extremely toxic to microorganisms (EC_{50} below 0.1 mg L^{-1}) and very toxic to algae (EC_{50} between 0.1 and 1 mg L^{-1}). Chronic toxicity tests performed on algae have shown high sensitivity to antibacterial agents as deduced from growth inhibition measurements. Vertebrates (such as fish) put directly in contact with low levels of antimicrobials apparently did not yield observable effects. Accordingly, a LC_{50} value above 100 mg L^{-1} for Japanese medaka concerning sulfonamides was reported.[34-38]

Numerous ecotoxicological studies were published in the last decade on the adverse effects of antibiotics on biological organisms, their toxicity, biovailability, hospital waste, biomarkers of oxidative stress and other environmental threaqts to ecosystems.[39-41] A recent toxicological problem is emerging with hospital effluent (containing among other residues, antibiotic as contaminants) that are collected in the same pipes as urban effluents (is called “coctail effect”) and they are mixed in the sewer. Thus, it is impossible to determine their specific contribution to global ecotoxicity or whether this mixture creates antagonistic or synergistic effects.[42]

Non steroidal anti-inflammatory drugs (NSAIDs) as emerging pollutants. Ecotoxicological studies have been contacted with non steroidal anti-inflammatory drugs in wastewater and other water sources. Organisms exposed to environmental concentrations of acetaminophen (AMP), diclofenac (DIC), ibuprofen (IBU), ketoprofen (KET) and nimesulide (NIM) revealed a significant accumulation of DIC, IBU and NIM, while AMP and KET were always below detection limit. Nonetheless, for all tested NSAIDs, measurement of a large panel of ecotoxicological biomarkers highlighted impairment of immunological parameters, onset of genotoxicity and modulation of lipid metabolism, oxidative and neurotoxic effects. Laboratory results were integrated with a field study which provided the first evidence on the occurrence of DIC, IBU and NIM in tissues of wild mussels sampled during summer months from an unpolluted, touristic area of Central Adriatic Sea. Overall results demonstrated that mussels *Mutilus galloprovincialis* can be used as a good sentinel species for monitoring presence and ecotoxicological hazard of pharmaceuticals in the Mediterranean Sea.[43]

Environmental pollution studies were carried out in seawater for NSAIDs and analgesics therapeutic classes. A recent study (2015, total 101 samples) in 14 sea beaches and five cities in North Portuguese coastal areas evaluated NSAIDs pollution. Acetaminophen, ketoprofen and the metabolite hydroxyibuprofen were detected in all the seawater samples at maximum concentrations of 584, 89.7 and 287 ngL^{-1}, respectively. Carboxyibuprofen had the highest seawater concentration (1227 ng L^{-1}). Analyses detected higher concentrations in August and September (bathing season). The environmental risk posed by the pharmaceuticals detected in seawaters towards different trophic levels (fish, daphnids and algae) was also assessed. Only diclofenac showed hazard quotients above one for fish, representing a potential risk for aquatic organisms. These results were observed in seawaters classified as excellent bathing water.[44]

Antineoplastic (anticancer) drugs in environmental studies. Environmental studies focused on cytostatic drugs as emerging toxic substances in water sources, especially discharged from hospitals to municipal wastewater treatment plants. A recent studiy analysed 14 cytostatic drugs in influent and effluent wastewater from

four wastewater treatment plants located in Seville (Spain) during 1-year period. A preliminary environmental risk assessment was also carried out. Five cytostatic drugs (cytarabine, etoposide, gemcitabine, iphosphamide, and methotrexate) were detected in influent wastewater at concentration levels up to 464 ng L^{-1} (cytarabine). Six of them (cytarabine, doxorubicin, gemcitabine, iphosphamide, paclitaxel, and vinorelbine) were detected in effluent wastewater at concentration levels up to 190 ng L^{-1} (cytarabine). Meassurements showed that these cytostatic drugs are not significantly removed during wastewater treatment. Researchers conclude that concentrations are very low for detectable ecotoxicological or genotoxical risks.[45]

The toxicological evaluation of the environmental impact of cytostatic drugs and their residues have been the subject of research by the European Union's 7th Water ramework Directive project "**CytoThreat**" This project was set up to investigate the effects of anticancer drugs in the environment and the need to develop new analytical methods and possible biomarkers for ecotoxicity data and environmental risk assessment [http://www.cytothreat.eu/index.php/results-and-impacts].[46]

A recent paper (2016) presented the results of acute toxicity involving 4 cytostatic drugs: 5-fluorouracil (5-FU), cisplatin (CDDP), etoposide (ET) and imatinib mesylate (IM) in zebrafish (*Danio rerio*) embryos and in adult fish and sub-chronic toxicity of 5-FU and IM in the early-life stage toxicity test. The results showed that at these low concetrations the cytostatic drugs tested were characterized by low acute and sub-chronic toxicity.[47]

The genotoxicity of cytostatic drugs is of great concern to environmentalists. The combined genotoxic effects of four anticancer drugs (5-fluorouracil [5-FU], cisplatin [CDDP], etoposide [ET], and imatinib mesylate [IM]) were studied testing their binary mixtures in two crustaceans that are part of the freshwater food chain, namely *Daphnia magna* and *Ceriodaphnia dubia*. Genotoxicity was assessed using the *in vivo* comet assay. The results obtained for *D. magna* showed independent action for all mixtures except for IM+5-FU that showed an antagonistic interaction. In *C. dubia*, most mixtures had antagonist interactions except IM+5-FU and

IM+CDDP that showed Bliss independence. Despite the antagonistic interactions, the results of the study demonstrated that combinations of anticancer drugs could be of environmental concern because effects occur at very low concentrations that are in the range of concentrations encountered in aquatic systems.[48]

Veterinary drugs and ecotoxicological studies. Veterinary drugs that are used in animal husbandry and in aquaculture (fish farming) have been proved to cause adverse effects (growth inhibition assays, inhibition of population growth, reproduction inhibition, luminescent inhibition) to aquatic organisms and subsequently to ecosystems. A recent study investigated the toxicity of 4 veterinary drugs: Doramectin (DOR), metronidazole (MET), florfenicol (FLO), and oxytetracycline (OXT) which are among the most widely used veterinary drugs. Scientists investigated their aquatic toxicity using tests with marine bacteria (*Vibrio fischeri*), green algae (*Scenedesmus vacuolatus*), duckweed (*Lemna minor*) and crustaceans (*Daphnia magna*). Ecotoxicological tests were supported by chemical analyses to confirm the exposure concentrations. Results (measured as Effective concentration 50%, EC_{50} in mg L^{-1}) found that OXT and FLO have a stronger adverse effect on duckweed (growth inhibition) and green algae than on bacteria (luminescent inhibition) and crustaceans (reproduction inhibition), whereas MET did not exhibit any adverse effect in the tested concentration range. For DOR a very low EC_{50} of 6.37 $\times 10^{-5}$ mg L^{-1} towards *D. magna* was determined, which is five orders of magnitude lower than values known for the toxic reference compound $K_2Cr_2O_7$. The results showed strong influence of these 4 veterinary drugs on representative aquatic organisms.[49]

Antibiotics in agriculture play a pivotal role in the management of infectious diseases in animal livestock and aquaculture operations at a global scale. But inevitably, veterinary drugs are released into the environment at an unprecedented scale causing concern for adversely impact in aquatic and terrestrial ecosystems. A recent critical review presented various research papers on ecotoxicological assessment of antibiotics as related to environmental risk assessment on microbial endpoints. For example, growth inhibition tests with cyanobacteria (*Cyanophyta*) (test OECD No 201) are required in Europe for both human and veterinary

antibiotics. Potential impacts of antibiotics on wastewater treatment are determined using the activated sludge respiration inhibition test (test OECD No 209). Also, ecotoxicity tests measure effects on soil microorganisms by using the soil nitrogen transformation (test OECD No 216) evaluating inhibition of the nitrification process. Also, ecotoxicity can be applied on single microbial species (e.g. the ISO 10712 *Pseudomonas putida* growth test).[50-52]

Ecotoxicological challenges with toxic pesticide residues

Despite numerous ecotoxicological studies in the past on the toxicity of pesticide residues, new research data are accumulating. The ecotoxicological assessment of pesticides has followed more detailed investigating developments which were stimulated by the unique hazardous properties of agricultural pesticides and subsequent influence on ecosystems. Within the discipline of ecotoxicology, considerable attention is directed towards the adverse impact of exposure to pesticides for non-targeted organisms and habitat disruption associated with their mode of usage, transformation, persistence, accumulation in lipd mebranes, biomagnification and genotoxicity.[53]

Figure 5. The global productive arable area has increased only by 10% in the last 50 years. It is estimated that 35% of the food produced in the world is lost to pests or wasted. Pesticides are playing an important role in the protection of agricultural production. The adverse health effects of pesticides to farmers are mainly cases of nmeglect and lack of safety and health protection during application.

Despite the hazardous properties of commercial pesticides, there is a need to take into account that the human population has more than doubled (7.4 billion) in the last 50 years and the global productive arable area has increased only by 10%.

According to FAO (Food and Agricultural Organization, http://www.fao.org/save-food/resources/keyfindings/en/) it is estimated that roughly 1/3 of the food produced in the world for human consumption every year (~1.3 billion tonnes) gets lost or wasted. Food losses and waste amounts to US$ ~680 billion in industrialized countries and US$ ~310 billion in developing countries. Fruits and vegetables, plus roots and tubers have the highest wastage rates of any food.

Although many efforts have been taken in the last decades to use biological means of pest control and other low toxicity methods, the increased use of chemical pesticides for the protection of agricultural crops and animals is still very important. Scientists often know a pesticide´s mode of action in the target species, but largely do not understand the full impact of unintended side effects on wildlife, particularly at higher levels of biological organization: populations, communities, and ecosystems. Many new ecotoxicological studies explore the toxicological spectrum of action to fill this gap in knowledge.[54] In this regard, a special issue of the specialised journal, Ecotoxicology , in 2017 is destined to collect a series of articles under the title“Emerging advances and challenges in pesticide ecotoxicology”.[55]

Another crucial problem of used pesticides is the emergence of pesticide resistance after a certain time of use. Scientists are working on various methods for resistance diagnosis for different groups of pests. A recent review provided an overview of biological, biochemical, and molecular methods that are currently used to detect and quantify pesticide resistance. Emerging technologies are also described.[56]

Scientists investigate lately some subtle toxic effects of pesticides. For example, a number of pesticide compounds have been proven to affect immune parameters in organisms, presenting cases of immunosuppression. Ecotoxicological studies showed that organochlorine pesticides, organophosphates, carbamates, atrazine, and 2,4-D were correlated to higher susceptibility of organisms to infection and parasite caused diseases. In mammals, the use of anticholinesterase agents in agriculture can pose a threat of infections, disease outbreaks, and higher mortality, such as by tularemia in hares.[57] Ecotoxicoilogical laboratory exposure experiments and field studies have shown an association between atrazine, malathion,

esfenvalerate, or glyphosate exposure and increased infection of tadpoles with trematodes.[58,59] A field survey of the northern leopard frog (*Lithobates pipiens or Rana pipiens*), revealed that atrazine pollution and inorganic phosphate accounted for 74% of the variation in the abundance of trematodes. Further mesocosm studies in ponds showed that atrazine killed the phytoplankton.[60]

An ecotoxicological study of pesticides was carried in 6 rivesrs and 10 lakes in Greece (1999-2001). The analytical technique of gas chromatography ion trap mass spectrometer was used for identification of 147 pesticides and their metabolites, (organochlorines, organophosphates, triazines, chloroacetanilides, pyrethroids, carbamates, phthalimides, etc). Results showed that the herbicides metolachlor, prometryn, alachlor and molinate, were the most frequently detected pesticides (often exceeding 1 μg/L) and chlorpyrifos ethyl was the most frequently detected insecticide. Annual average concentrations of chlorpyriphos ethyl (0.031 μg/L), dicofol (0.01 μg/L), dieldrin (0.02 μg/L) and endosulfan a (0.065 μg/L) exceeded the EU environmental quality standards. The risk quotient estimates for the insecticides chorpyrifos ethyl, diazinon and parathion methyl and herbicide prometryn were above acceptable risk values.[61]

In the last decade the ecotoxicological assessment of pesticides followed the biomarkers approach in order to study more effectively the unique properties of toxic residues and damaging effects on specific biological targets. Within the discipline of ecotoxicology, considerable attention was directed towards the adverse impact of exposure to tailored pesticides with an increased apprehension on the broadening effects for non-targeted organisms and habitat disruption associated with their usage.[62] Adverse impact of pesticides and metabolites on wildlife, environmental biodiversity and ecosystem structure and function were particularly investigated by recent ecoltoxicological projects.[63-65]

In 2007, an international database was launched Pesticide Properties Database (PPDB) as a free-to-access website and has continued to expand. It is referred also as the FOOTPRINT database because some work on the database is being undertaken as part of the FOOTPRINT EU FP-6 project. Currently, this database (PPDB) holds data for almost 2,300 pesticide active substances (synthetic and

natural, including those with veterinary applications) and over 700 records for associated metabolites. The database is used extensively throughout the world, endorsed by the International Union of Pure and Applied Chemistry (IUPAC) and promoted by several major organizations including the FAO. Various on-line databases are also consulted, including the European Chemical Substances Information System (ECSIS) for chemical identification data, the U.S. National Institutes of Health databases for chemical identification and toxicology, and the International Uniform Chemical, Information Database for information on chemical hazards: Also, the U.S. ECOTOX database is used as a source for ecotoxicity data (http://cfpub.epa.gov/ecotox/). Over the last 20 years, this database has been keeping pace with improving risk assessments, their associated data requirements, and the needs and expectations of database end users. For each pesticide substance around 300 parameters are stored, covering human health, environmental quality, bioaccumulation, effects on plants and biodiversity risk assessments.[66]

Ecotoxicological studies with neonicotinoid pesticides

Neonicotinoid pesticides were for many decades very popular with farmers, a new class of pesticides produced in significant quantities (imidacloprid, acetamiprid, thiamethoxam, etc.) act specifically to the nicotinic acetylcholine receptor (nAChR) of insects. Neonicotinoids and fipronil currently account for approximately one third (in monetary terms in 2010) of the world insecticide market. They are applied in many ways, including seed coating, bathing, foliar spray applications, soil drench applications and trunk injection. These compounds are used for insect pest management across hundreds of crops in agriculture, horticulture and forestry. They are also widely used to control insect pests and disease vectors of companion animals, livestock and aquaculture and for urban and household insect pest control and timber conservation.[67]

In 2012 "**The Task Force on Systemic Pesticides**" (TFSP) was the response of the scientific community to concern around the impact of systemic pesticides on biodiversity and ecosystems. TFSP's intention was to provide the definitive view of science to inform more rapid and improved decision-making. The mandate of the

Task Force on Systemic Pesticides (TFSP) has been set by IUCN (International Union for Conservation of Nature) Resolution WCC-2012-Res-137.[68]

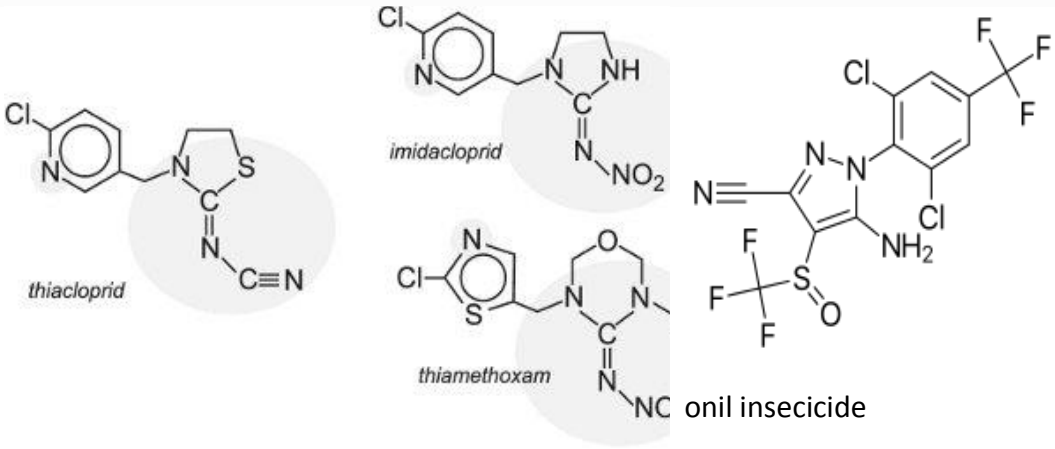

Figure 6. Neonicotinoids (Thiacloprid, Imidacloprid, Thiamethoxam) and Fibronil insecticides. Recent studies with neonicotinoid insecticides under field-realistic conditions detected harm to honeybee colonies and their reproduction.

Support for a comprehensive scientific review of the impact on global biodiversity of systemic pesticides by the joint task force of the IUCN Species Survival Commission (SSC) and the IUCN Commission on Ecosystem Management (CEM), adopted by the Members' Assembly of the IUCN in Jeju, Korea, in September 2012: The Worldwide Integrated Assessment (WIA) of the impact of systemic pesticides on biodiversity and ecosystems has made a synthesis of 1,121 published peer-reviewed studies spanning the last five years, including industry-sponsored ones. It is the single most comprehensive study of neonics ever undertaken, is peer reviewed, and published as open access so that the findings and the source material can be thoroughly examined by others. Some aspects of this analysis have been broadly acknowledged before (e.g. risks to honeybees), but some have not (e.g. risks to birds, earthworms, other pollinators and aquatic invertebrates) [http://www.tfsp.info/worldwide-integrated-assessment/].[68]

A recent review summarized the results and conclusions of "The Worldwide Integrated Assessment (WIA)" (2015) that was the first attempt to synthesize the state of knowledge on the risks to biodiversity and ecosystem functioning posed by the widespread global use of neonicotinoids and fipronil. The WIA was based on the results of over 800 peer-reviewed scientific journal articles published over the past

two decades. The review assessed respectively the trends, uses, mode of action and metabolites of neocotinoids and fibronil, the environmental fate and exposure. Neocotinoids and the effects on non-target invertebrates, direct and indirect effects on vertebrate wildlife; and risks to ecosystem functioning and services and finally explored sustainable pest management practices that can serve as alternatives to the use of neonicotinoids and fipronil.[68-71]

Although neonicotinoids are considered low toxicity to mammals and humans in comparison with traditional insecticides, more and more studies show exposure to neonicotinoids pose potential risk to mammals and even humans. Owing to the environmental pollution of neonicotinoids, a series of apparent bee-poisoning events happened in Europe. In 2013, the European Union temporarily prohibited the use of clothianidin, imidacloprid, and thiamethoxam insecticides. However, neonicotinoids have been widely used in Latin America, Asia, and North America, accounting for 75% of total global sales.[72] Neonicotinoids have been detected in human urine, serum and hair. In *vivo* and *in vitro* studies suggested that neonicotinoids are toxic.[73]

Most early studies of the adverse effects of neonocotinoid insecticides on insect pollinators (honeybees) indicated considerable harm, but most criticisms of these investigations rested on the fact that they did not represent field-realistic conditions. Studies conducted on different crops and on two continents showed that neonicotinoids diminish bee health, especially corn crops exposed to insecticide for 3-4 months. A multicountry experiment on rapeseed in Europe found that neonicotinoid exposure for several nontarget sources reduced overwintering success and colony reproduction in both honeybees and wild bees under realitic agricultural conditions.[74]

Roundup (Glyphosate) the most widely used herbicide

Glyphosate is an herbicide. It is applied to the leaves of plants to kill both broadleaf plants and grasses. The sodium salt form of glyphosate is used to regulate plant growth and ripen fruit. Glyphosate was first registered for use in the U.S. in 1974 by (Monsanto, commercial name Roundup) and is one of the most widely used herbicides in the USA and other countries. It is used in agriculture and

forestry, on lawns and gardens, and for weeds in industrial areas. Glyphosate comes in many forms, including an acid and several salts. There are over 750 products containing glyphosate. Glyphosate was adopted especially after Monsanto introduced glyphosate-resistant Roundup Ready crops, enabling farmers to kill weeds without killing their crops. In 2007, glyphosate was the most used agricultural herbicide in the USA and second-most used in home and gardens. By 2016 there was a 100-fold increase in the frequency of applications.[75]

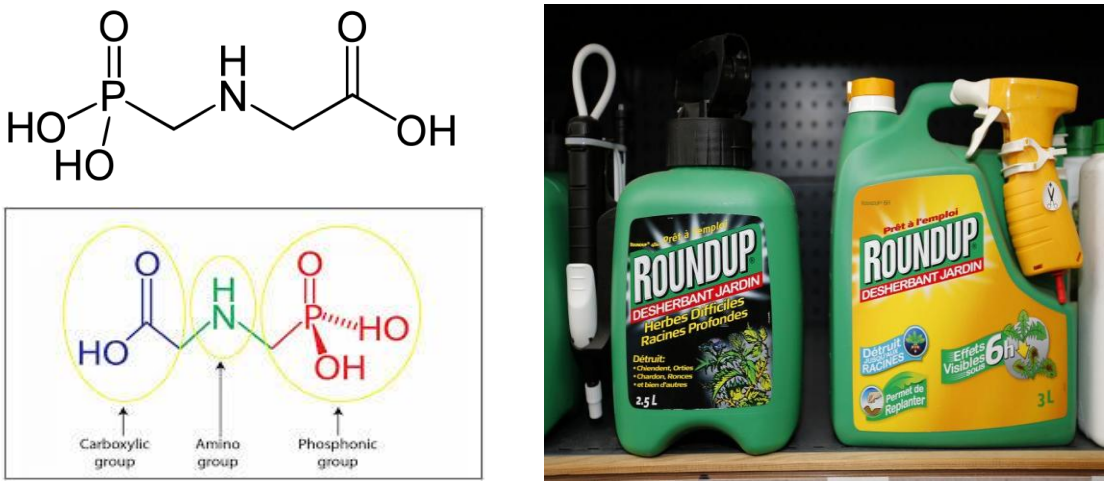

Figure 7. Glyphosate (Monsanto, Roundup), is considered an effective weeds killing pesticide and the most used herbicide by farmers in the USA.

Glyphosate and formulations have been approved by regulatory bodies worldwide, despite concerns about their adverse effects on humans and the environment. Also, many regulatory and scholarly reviews have evaluated the relative toxicity of glyphosate. The German Federal Institute for Risk Assessment (Bundesintitut fur Risikobewertung) toxicology review in 2013 found that "the available data is contradictory and far from being convincing" with regard to correlations between exposure to glyphosate formulations and risk of various cancers, including non-Hodgkin lymphoma. BfR is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany and advises the Federal Government and Federal Laender on questions of food, chemical and product safety.[76,77]

In 2014 a meta-analysis (quantitative assessment) was published with a series of research studies that showed an increased risk of Non-Hodgkin lymphoma in workers exposed to glyphosate formulations.[78,79]

A Working Group of 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC) on 3-10 March 2015 to review the available published scientific evidence and evaluate the carcinogenicity of five organophosphate insecticides and herbicides: diazinon, glyphosate, malathion, parathion, and tetrachlorvinphos. The International Agency for Research on Cancer (IARC, Lyon, 2015) taking into account epidemiological studies, in vivo studies (animals), and *in vitro*s tudies (cell cultures) classified glyphosate as "probably carcinogenic in humans" (category 2A). The report "….for the herbicide glyphosate, there was limited evidence of carcinogenicity in humans for non-Hodgkin lymphoma". The evidence in humans is from studies of exposures, mostly agricultural, in the USA, Canada, and Sweden published since 2001. In addition, there is convincing evidence that glyphosate also can cause cancer in laboratory animals. On the basis of tumours in mice, the USA EPA) originally classified glyphosate as possibly carcinogenic to humans (Group C) in 1985. After a re-evaluation of that mouse study, the US EPA changed its classification to evidence of non-carcinogenicity in humans (Group E) in 1991. The US EPA Scientific Advisory Panel noted that the re-evaluated glyphosate results were still significant using two statistical tests recommended in the IARC Preamble. The IARC Working Group that conducted the evaluation considered the significant findings from the US EPA report and several more recent positive results in concluding that there is sufficient evidence of carcinogenicity in experimental animals. Glyphosate also caused DNA and chromosomal damage in human cells, although it gave negative results in tests using bacteria. One study in community residents reported increases in blood markers of chromosomal damage (micronuclei) after glyphosate formulations were sprayed nearby.[80,81]

In November 2015, the European Food Safety Authority (EFSA) published an updated assessment report on glyphosate, concluding that "the substance is unlikely to be genotoxic (i.e. damaging to DNA) or to pose a carcinogenic threat to humans."

The final report clarified that while other, probably carcinogenic, glyphosate-containing formulations may exist, studies "that look solely at the active substance glyphosate do not show this effect."The WHO and FAO Joint committee on pesticide residues issued a report in 2016 stating that the use of glyphosate formulations does not constitute a health risk and also gave admissible daily intake limits for chronic toxicity. The European Chemicals Agency (ECHA) maintained their current classification of glyphosate as a substance causing serious eye damage and as a substance toxic to aquatic life, but did not find evidence implicating it to be a carcinogen, a mutagen, toxic to reproduction, nor toxic to specific organs.[82-85]

In September 2017, articles appeared in a number of European press outlets casting doubt on the integrity of the EU assessment of Glyphosate, in particular the content of the assessment report submitted to EFSA by the German Federal Institute for Risk Assessment (BfR). EFSA responded with a statement in which it defended the robustness of the EU assessment and pointed out that the allegations were based on a misunderstanding of the peer review process. On July 6 2017, upon request from the European Commission, EFSA and the European Chemicals Agency (ECHA) replied to a letter from Professor Christopher Portier to President Juncker regarding their evaluation of the carcinogenicity of Glyphosate. On June 8 2017 EFSA published a statement concerning the EU assessment of glyphosate following allegations made in the so-called “Monsanto papers”. The statement, which was requested by the European Commission, outlines the EU legislative framework concerning the submission of open scientific literature for the assessment of active substances and explains how such literature is considered by EU Member States and EFSA experts during the peer-review process [EFSA 2017, Glyphosate https://www.efsa.europa.eu/en/topics/topic/glyphosate].

Meanwhile, farmers in United Kingdom (UK), France and Germany started worrying that banning Glyphosate will remove an important tool in conservation farming, using minimum tillage and building carbon in soils. Advice from rural environmental consultancy ADAS stated Glyphosate adds $1 billion in value to UK farm production with increased crop yields and pastures. In Germany, farmers have

campaigned to keep the herbicide, using roadside signs declaring "harvests in danger".[86]

The European Commission (October 2017) ordered the re-authorisation of Glyphosate for another 5 years. Glyphodsate products (herbicides) were the subject to a heated debate in Brussels. A consensus was reached on the 27/12/2017 for permission to use Glyphosate in the 28 countries of the European Union for another 5 years. (Europarc, Glyphosarte debate in Brussels, 26.10.2017 http://www.europarc.org/news/2017/12/glyphosate-debate/].

Disinfection by-products and toxicological studies

There is widespread potential for human exposure to disinfection by-products (DBPs) in drinking water because everyone drinks, bathes, cooks, and cleans with municipal water. Chlorine (Cl_2) has been widely used for decades worldwide as a chemical disinfectant, serving as the principal barrier to microbial contaminants in drinking water. Current studies indicate that using or drinking water with small amounts of chlorine does not cause harmful health effects and provides effective protection against waterborne disease outbreaks. The noteworthy biocidal attributes of chlorine have been somewhat offset by the formation of disinfectant by-products (DBPs) of public health concern during the chlorination process. As a consequence, alternative chemical disinfectants, such as ozone (O_3), chlorine dioxide (ClO_2) and chloramines (NH_2Cl, monochloramine), are increasingly being used. However, every disinfectant has been shown to form its own set of DBPs. Although the microbiological quality of drinking water cannot be compromised, there is a need to better understand the chemistry, toxicology and epidemiology of chemical disinfectants and their associated DBPs in order to develop a better understanding of the health risks (microbial and chemical) and to seek a balance between microbial and chemical risks. It is possible to decrease the chemical risk due to DBPs without compromising microbiological quality.[87, 88]

Disinfection by-products are non-carcinogenic according to World Health Organization (WHO) at the low concentrations used**.** In 1991 the WHO's International Agency for Research on Cancer (IARC) evaluated the carcinogenic

health risk of chlorinated drinking water based on toxicological laboratory studies and human epidemical researches. This study showed that it is hard to find a relation between the development of cancer and drinking of chlorinated water. The risk is small and cannot be proved with epidemiological evidence. Epidemiological studies assessing health risks over consumption of drinking water with disinfection by-products observed small carcinogenic risks.[89-91]

While protecting against microbial contamination is the top priority (for human health), municipal water systems must also control disinfection by-products (DBPs), chemical compounds formed unintentionally when chlorine and other disinfectants react with natural organic matter in water. In the early 1970s, EPA scientists first determined that drinking water chlorination could form a group of by-products known as trihalomethanes (THMs), including chloroform. EPA set the first regulatory limits for THMs in 1979. While the available evidence does not prove that DBPs in drinking water cause adverse health effects in humans, high levels of these chemicals are certainly undesirable. The health risks from these by=products at the levels at which they occur in drinking water are extremely small in comparison with the risks associated with inadequate disinfection [Water and Health https://waterandhealth.org/safe-drinking-water/wp/].

The environmental toxicity of DBPs increases substantially for higher concentration uses in sewer treatment. Alternative chemical disinfectants were investigated with lower environmental toxicity which can affect aquatic species. Many disinfectants degrade quickly in water which should be included in the evaluation of both their toxicity as determined in standardized tests and their possible negative effect in the water environment. A recent study evaluated (standardized ISO 8692 test) the toxicity of 3 disifenctants towards the green microalgae *Pseudokirchneriella subcapitata.* These were a)performic acid (PFA), b) peracetic acid (PAA) and c) chlorine dioxide (ClO_2)] as well as two by-products of their use d) hydrogen peroxide (H_2O_2) and e) chlorite. All of the five chemicals investigated showed clear toxicity to the algae with well-defined dose response curves. The EC_{50} values ranged from 0.16 to 2.9 mg/L. The 5 investigated chemicals decreased in toxicity in the order chlorine dioxide, performic acid, peracetic acid,

chlorite and hydrogen peroxide. The stability of the chemicals increased in the same order as the toxicity decrease. This indicates that even though ClO_2 has the highest environmental hazard potential, it may still be suitable as an alternative disinfectant due to its rapid degradation in water.[92]

Several disinfectant by-products (DBPs) have been implicated in a variety of ecotoxic effects, including developmental effects in fish. An ecotoxicological study investigatedand evaluated the developmental toxicity and genotoxicity of 10 DBPs (four trihalomethanes [THMs], five haloacetic acids [HAAs] and sodium bromate) in the zebrafish embryo model (exposed for 72 hours, endpoints growth, hatching success, malformations and lethality). THMs exposure resulted in adverse developmental effects and a significant reduced tail length. Two HAAs, tribromoacetic acid and dichloroacetic acid, along with sodium bromate were found to cause a significant increase in malformation rate. Chloroform, chlorodibromomethane and sodium bromate produced a weak induction of DNA damage to whole embryos. However, developmental effects occurred at a range of concentrations (20–100 μg/mL) several orders of magnitude (much higher than normal pollution concentrations) above the levels that can be attained in fetal blood in humans exposed to chlorinated water. Researchers concluded that the teratogenic and genotoxic activity observed by some DBPs in zebrafish reinforce the view that there is a weak capacity of of disinfection products to cause developmental effects at environmentally relevant concentrations.[93]

Ecotoxicology of wood preservation products

Wood preservatives are biocidal products used to protect wood building materials from wood destroying or wood-disfiguring organisms. Active ingredients such as metallic salts, quaternary ammonium salts, carbamates and azoles are frequently employed. Solid timber or wood based products are used for diverse service situations and are therefore classified according to different "Use Classes", as defined by the European standard EN 335: 2013. Situations for which wood product is above ground and exposed to the weather or in direct contact with ground are

classified under use class 3 and 4, respectively. In this case, the chemical substances employed are likely to reach the soil compartment either by leaching from the treated wood surface or by direct contact between the wood and the soil. They may then pose a potential risk for the soil organisms, the essential functions they performed and thus for the soil ecosystem in general. Currently, the available toxicity data for terrestrial organisms regarding the active ingredients present in wood preservatives are still scarce.[94]

Studies for the evaluation of ecotoxicity in soil organisms were conducted in 2015 by the Centre Ecotox [Centre Suisse d'écotoxicologie appliquée, Eawag-EPFL, 1015 Lausanne] for 4 wood preservatives: a. IPBC (Iodopropynyl butylcarbamate), b) Propiconazole, c) Boric acid and Copper(II)hydroxide, with Chromium as fixing agent; referred to as "CuCrB" and d) Copper(II)carbonate-Copper(II)hydroxide, Didecylpolyoxethylammoniumborat (DPAB) and Boric acid; referred to as "Quats". The ecotoxicity results showed that IPBC induced toxic effects in the same order of magnitude for both collembolans and earthworms. These effects are also in the same range of toxicity than effects reported in the literature for other carbamate compounds. The EC_{50} value found for *E. andrei* avoidance behaviour is far lower than the acute toxicity value reported for earthworms in the PT8 assessment report for IPBC. This result underlines the importance of considering other and more sensitive endpoints than earthworm survival in risk assessment. Earthworms reacted to concentrations of Propiconazole that are in the same order of magnitude than effect concentrations found for IPBC. However, they showed to be slightly more sensitive than collembolans to this compound. Our toxicity values are in the range of toxicity reported for soil organisms for Propiconazole in the PT8 assessment report. Both CuCrB and Quats wood preservatives contained a mix of active ingredients and therefore showed a quite high toxicity for collembolans but even more important for earthworms. The toxicity induced by the mixture of active ingredients is far below the toxic effects observed for the individual substance itself. Chromium seems to participate to a large extend of the toxicity of CuCrB to earthworms and collembolans. It is however not considered as an active ingredient in the CuCrB products and not always taken into account in leaching studies. Different toxicity

may arise depending on its oxidation state and have to be considered. Soil chemical analysis should be run in parallel to the conducted ecotoxicity tests to define the type of chromium present, but also to inform on the available fraction of the active ingredients present in the soil matrix during the assays. DPAB do not seem to be highly toxic neither for collembolans nor for earthworms. Based on the obtained results and in regards to the available earthworm toxicity data found in the literature, earthworm avoidance behaviour can be considered as an appropriate and sensitive endpoint to assess toxicity of biocidal substances.[94]

Various ecotoxicological studies were performed with soil invertebrates and the chronic toxicity of Chromium (III) was assessed for *Eisenia fetida*, *Enchytraeus albidus*, and *Folsomia candida*, the three invertebrates for which standard test protocols are available.[95,96]

Biocides are common additives in building materials. Film preservatives in polymer-resin render and paint, as well as wood preservatives are used to protect facade materials from microbial spoilage. Biocides leach from the facade material with driving rain, leading to highly polluted runoff water (several mg L^{-1} biocides) being infiltrated into the soil surrounding houses. A study investigated the degradation rates in soil of 11 biocides used for the protection of building materialsin tests with laboratory microcosms. The results of the study showed that some biocides are degraded rapidly in soil (e.g., isothiazolinones: $T_{1/2}$ < 10 days) while others displayed higher persistence (e.g., terbutryn, triazoles: $T_{1/2} \gg 120$ days). Microtox-tests revealed that degradation of preservative products were less toxic toward the bacterium *Aliivibrio fischeri* than their parent compounds.[97]

Ecotoxicology of environmental pollution by flame retardants

For decades, foam furniture, baby products and electronics have been loaded with chemicals which acted as flame retardants. Most of these chemicals were polybrominated and polychlorinated compounds have been linked to cancer and hormone disruption, as well as deficits in motor skills, attention and IQ in children. Though the most toxic ones have been phased out in the United States, they were replaced with poorly studied alternatives that also could harm health.

In 2017, the Consumer Products Safety Commission (CPSC) initiated a ban on the most toxic additives in foam products and electronics, and warned the public, particularly parents, to avoid buying new foam or electronic products that contain bromine- or chlorine-based flame retardants. Brominated flame retardant chemicals, banned in the U.S. since 2004, still pollute the bodies of newborn American babies, according to a new study from Indiana University scientists. Polybrominated diphenyl ethers (PBDEs) were once widely used in products *including* furniture foam and electronics. Exposure to PBDEs is linked to learning, memory and developmental problems, as well as endocrine disruption and cancer in both animal and epidemiological studies. Brominated flame retardants (BFRs) have been routinely used as additives in a number of consumer products for several decades in order to reduce the risk of fire accidents. Concerns about the massive use of these substances have increased due to their possible toxicity, endocrine disrupting properties and occurrence in almost all the environmental compartments, including humans and wildlife organisms. Over the past few years, these compounds have been replaced with "new" BFRs (NBFRs). Despite the fact that NBFRs are different chemical molecules than the BFRs, most of physical–chemical properties (e.g. aromatic moiety, halogen substitution, lipophilic character) are common to both groups; therefore, their fate in the environment is potentially similar to the banned BFRs.[98]

Toxicological studies of PBDEs and PCBs have been contacted on seawater musselsBaltic. In 1999, sea blue mussels, *Mytilus edulis*, were exposed to polybrominated diphenyl ethers (PBDEs, IUPAC congeners 47, 99, and 153) and polychlorinated biphenyls (PCBs, congeners 31, 52, 77, 118, and 153) in a flow-through experimental setup for 44 days. After the exposure phase, the mussels were allowed to depurate in natural brackish water for 26 days. The results showed that the bioaccumulation potential of PBDEs (used as flame retardants), is similar or higher than that of PCBs for filter feeding organisms such as blue mussels.[99]

A toxicological study evaluated the neurobehavioral effects of acute or developmental exposure in zebrafish for selected new flame retardants (which were introduced recently as potential commercial replacement of flame retardants in consumer and electronic products). The f.r. were *t*-butylphenyl diphenyl phosphate

(BPDP), 2-ethylhexyl diphenyl phosphate (EHDP), isodecyl diphenyl phosphate (IDDP), isopropylated phenyl phosphate (IPP), tricresyl phosphate (TMPP; also abbreviated TCP), triphenyl phosphate (TPHP; also abbreviated TPP), tetrabromobisphenol A (TBBPA), tris (2-chloroethyl) phosphate (TCEP), tris (1,3-dichloroisopropyl) phosphate (TDCIPP; also abbreviated TDCPP), tri-*o*-cresyl phosphate (TOCP), and 2,2-,4,4'-tetrabromodiphenyl ether (BDE-47) in zebrafish (*Danio rerio*) larvae at sub-teratogenic concentrations either developmentally or acutely, and locomotor activity was assessed at 6 days post fertilization. The results of this study indicated that these replacement flame retardants may have developmental or pharmacological effects on the vertebrate nervous system.[100]

Plasticizers and their metabolites in ecotoxicological investigations

Plasticizers are additives used to increase the flexibility or plasticity, of polymers. Some of the common plasticizers were recognized as endocrine disruptors, toxic to animals and some have been banned or strictly regulated. Plasticizers are emerging pollutants which are measured in sewage treatment plant effluents. The discovery of plasticizers transformed the polymer industry. Without a plasticizer, most polymers would just be too brittle and rigid to be useful. Virtually any plastic or polymer is used for commercial products has a plasticizer added to it, and often more than one. Phthalates are used as plasticizers in PVC cables, films, coatings, adhesives and certain plastics that need flexibility, dicarbonates are also used as plasticizers in PVC when the polymer needs to work in low temperatures. Phosphate plasticizers add flame-retardant qualities to polymers and fatty acid esters plasticizers impart flexibility to rubber and vinyl polymers. The current legislative and toxicological status of plasticizers are thoroughly reviewed to provide information about the environmental effects of this widespread use of these products.[101]

Ecotoxicological studies on the toxicity of plasticisers Phthalate Esters (PAEs) have focused on growth response of terrestrial and aquatic animals, but only limited attention has been paid to aquatic plants (phytoplankton, the primary producer in aquatic ecosystems). A recent study (2017) focused on the acute toxic effects

(growth) of the plastizer Dibutyl Phthalate (DBP) at different concentrations (0–20 mg/L) on two typical freshwater algae (*Scenedesmus obliquus* and *Chlorella pyrenoidosa*). The results showed that the growth of both algae were conspicuously inhibited. Damage occurred to cell organelles and chlorophyll content conspicuously decreased. Algal growth inhibition was closely linked to the increased production of intracellular reactive oxygen species (ROS) and malondialdehyde content, indicating oxidative stress and lipid peroxidation in both algae.[102]

Plastic additives to improve polymer properties have attracted a number of research, because as adsorbed pollutants on plastic pieces (microplastics) can be found worldwide in the aquatic environment. These chemical plasticizers can leach out of the polymer materials since some are not chemically bound. As a consequence of plastic accumulation and fragmentation in oceans, plastic additives (plasticizers, colouring material, antioxidants) could represent an increasing ecotoxicological risk for marine organisms. A review investigated a number of studies for the main class of plastic additives identified in the literature, their occurrence in the marine environment, as well as their effects on and transfers to marine organisms. The additives identified were: polybrominated diphenyl ethers (PBDE), phthalates, nonylphenols (NP), bisphenol A (BPA) and antioxidants as the most common plastic additives found in marine environments. Resarch showed that these plastic additives are leached and absorbed by marine organisms, with experiments in laboratory and environmental field studies. Scientists suggested that future research must focuse on the toxicity of microplastics and plastic additives as potential hazards for marine organisms. A greater focus on the transport and fate of plastic additives is now required because additives may easily leach out from plastics in the aquatic environment.[103]

Ecotoxicology of polyfluorinated compounds,

Polyfluorinated and perfluorinated (PFCs) are molecules made up of carbon chains (C-C-C-) to which fluorine (F) atoms are bound. Due to the strength of the (C–F) carbon/fluorine bond, the PFC molecules are chemically very stable and highly resistant to biological degradation. Perfluorinated compounds [PFCs] and

polyfluorinated substances have found a wide use in industrial products and processes and in a vast array of consumer products. These compounds can bioaccumulate and also undergo biomagnification. Within the class of PFC chemicals, perfluorooctanoic acid (PFOA) and perfluorosulphonic acid (PFOSA) are generally considered reference substances. Meanwhile, PFCs can be detected almost ubiquitously, e.g., in water, plants, different kinds of foodstuffs, in animals such as fish, birds, in mammals, as well as in human breast milk and blood. PFCs are proposed as a new class of "persistent organic pollutants".[104,105]

The presence of PFCs in high-mountain lakes, deep-ocean and offshore waters in a wide range of geographical locations have been investigated in recent years giving the opportunity to scientists to understand the global distribution of PFCs in aquatic organisms. High concentrations of PFCs continue to be detected in recent years in invertebrates, fish, reptiles and marine mammals worldwide. Perfuorooctane sulfonate (PFOS) is still the predominant PFC detected (mean concetr. 1900 ng/g ww). Also, long-chain perfuoroalkyl carboxylates are detected (PFCAs mean concentr. 400 ng/g ww). A number of studies have evaluated the bioaccumulation and biomagnification of PFCs in freshwater and marine food webs. Several studies have reported a decrease in PFOS levels over time, in contrast to PFCA that tended to increase in tissues of aquatic organisms in many locations.[106]

Ecotoxicology of cyanotoxins and cyanobacteria of eutrophication

Eutrophication of water bodies from municipal effluents and global warming are the factors are contributing to algal blooms. Massive proliferations of cyanobacteria in freshwater, brackish and coastal marine ecosystems have become a worldwide environmental problem. Anthropogenic eutrophication (input of nutrients, especially phosphorous and nitrogen) of surface waters leads to accelerated growth of photoautotrophic organisms including cyanobacteria. As a consequence, cyanobacteria have the ability to form a great variety of several secondary metabolites, which exhibit various types of biological or biochemical activities and some of them have been identified as potent toxins (cyanotoxins). The cyanotoxins are a diverse group of compounds, both from the chemical and the

toxicological points of view. In terms of their toxicological target, cyanobacterial toxins are hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins.[107, 108]

The development in coastal waters or inland aquatic areas of harmful algal blooms (HAB) results in restrictions on fisheries, recreational activitiews, and use of drinking water. Harmful algal bloom impacts in the aquatic environment are not as predictable as those from conventional chemical contaminants, because interactions among multiple natural and anthropogenic factors determine the likelihood and severity (depending on toxicity of cyanotoxins released) to which a HAB will occur. Harmful algal blooms present significant challenges for achieving water quality protection and restoration goals. The investigation of cyanobacteria blooms need expertise on environmental toxicology and chemistry, and risk-assessment expertise from ecologists, engineers and public health practitioners.[109]

In a recent ecotoxicological study, scientists isolated strains of the cyanobacteria *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*, which produce microcystin (MC) and saxitoxin (STX), respectively. Ecotoxicological tests using suspensions of lysed lyophilizated cells with concentrations of toxins equivalent to those permitted by legislation for potability (1 µg/L for MC and 3 µg/L for STX) did not result in significant mortality of the model organism, *Ceriodaphnia dubia*, where as concentrations five times greater resulted in decreased survival for both toxins. However, reproduction was significantly reduced even in the lower concentrations, indicating that the currently permitted standards are not safe for environmental protection. When cyanotoxins were treated with ultrasound, mortalities were no longer significant, independent of concentrations. Although reproduction was still lower in relation to the control, it was significantly higher when compared to the results obtained before ultrasound. Ultrasound has been previously applied to cyanobacteria cell lysis, but this is the first study to investigate the ecotoxicological effects of ultrasound on cyanotoxins. [110]

It is well known that cyanobacterial blooms pose serious threats to the functioning of aquatic ecosystems because they produce a wide range of potentially bioactive secondary metabolites (cyanotoxins). A recent

ecotoxicological study provided proteomic and metabolomic analyses to evaluate the global response of hundreds of proteins and metabolites at a glance. Medaka fish (*Oryzias latipes*) were exposed for 96 hours either to a microcystin (MC)-producing or to a non-MC-producing strain of *Microcystis aeruginosa*and cellular, proteome and metabolome changes following exposure to cyanobacteria were characterized in the fish livers. The results of the study suggest that a short-term exposure to cyanobacteria, producing or not MCs, induces sex-dependent molecular changes in medaka fish, without causing any cellular alterations. Globally, molecular entities involved in stress response, lipid metabolism and developmental processes exhibit the most contrasted changes following a cyanobacterial exposure.[111]

Synthetic musks fragrances in toxicological research

Synthetic musk fragrances are common additives in personal care products such as soaps, lotions, deodorants, antiseptics, and detergents. Their presence was first detected in the environment in 1981 and they have since been detected in nearly all environmental compartments including water, sediment, aquatic organisms, and humans. They are released into the environment almost entirely as a result of wastewater discharges.[112-114]

Personal care products (PCPs), such as synthetic musk fragrances, cosmetic creams, disinfectants, purfumes, insect repellents, preservatives and UV filters, are used extemsively in the last decades. Some of them are considered chemicals of emerging concern due to their presence and negative impact on aquatic ecosystems, specially related to endocrine disruption and reproductive disorders in marine organsims. The entry of those chemicals to water bodies occurs mainly through the sewage effluents from wastewater treatment plants due to their incomplete or inefficient removal. A recent review collected and analyzed research articles on concentrations of PCPs in different water matrices and wastewater effluents. The database contained 141 articles with information about 72 PCPs recorded as emerging pollutants in 30 countries, in concentrations ranging from 0.029 ng/L to 7.811×10^6 ng/L. Fragrances, antiseptics and sunscreens were the most reported

groups. Most of PCPs were found in wastewater treatment plant effluents (total 64 compounds), compared to 43 in surface water and 23 in groundwater. These molecules were found in all the continents.[115]

In the last decade much research has been focused on the endocrine-disrupting potential of Pharmaceuticals and Personal Care Products (P&PCPs) and their impact on aquatic organisms. A review (2015) assessed the results on the reported effects of PPCPs on fish reproduction (fecundity). The majority of individual P&PCP studies reviewed observed negative effects on fish fecundity. In addition, laboratory and field assessments of wastewater treatment plant (WWTP) were reviewed and the results from were variable. In general, they demonstrated negative impacts on reproduction. Also, the studies are unable to directly associate observed adverse effects with WWTP effluents. [116]

Ecotoxicological studies of nanoparticle and nanomaterials

Nanoparticles are new engineered materials that were introduced in the last decade in many commercial products. Nanomaterials are chemical-based very small sized materials of which a single unit is sized between 1 to 1,000 nanometres (10^{-9} meter). There are many types of nanoparticles. Some nanomaterials are nanocrystals made of metals, semiconductors, or oxides with particular interest for their mechanical, electrical, magnetic, optical, chemical and other properties. In the last decade nanoparticles have been used as quantum dots and as chemical catalysts. A range of nanoparticles have been extensively investigated for biomedical applications including tissue engineering, drug delivery and biosensors. Nanoparticles are of great scientific interest because of their specific properties and are effectively a bridge between bulk materials and atomic and molecular structures.[117,118]

Despite their worldwide advancement into everyday products, nanomaterials and nanoparticles present health and safety risks for human health (industrial workers and consumers), the environment and wildlife ecosystems. Among scientists there is a wider debate about the risks and benefits of the many engineered nanomaterials in consumer products. The ecotoxicology scientific community is only

at the beginning of understanding the potential risks to wildlife associated with manufactured nanomaterials. Toxicologists just now recognise that these nanomaterials may have unusual physico-chemical properties, or behaviours in water (e.g., colloid chemistry), and present exceptional risks compared to other chemical pollutants. In this respect, engineered nanomaterials are considered a subject of urgent investigation as unusul emerging pollutants with specific health and safety problems for humans and the environment.[119-121]

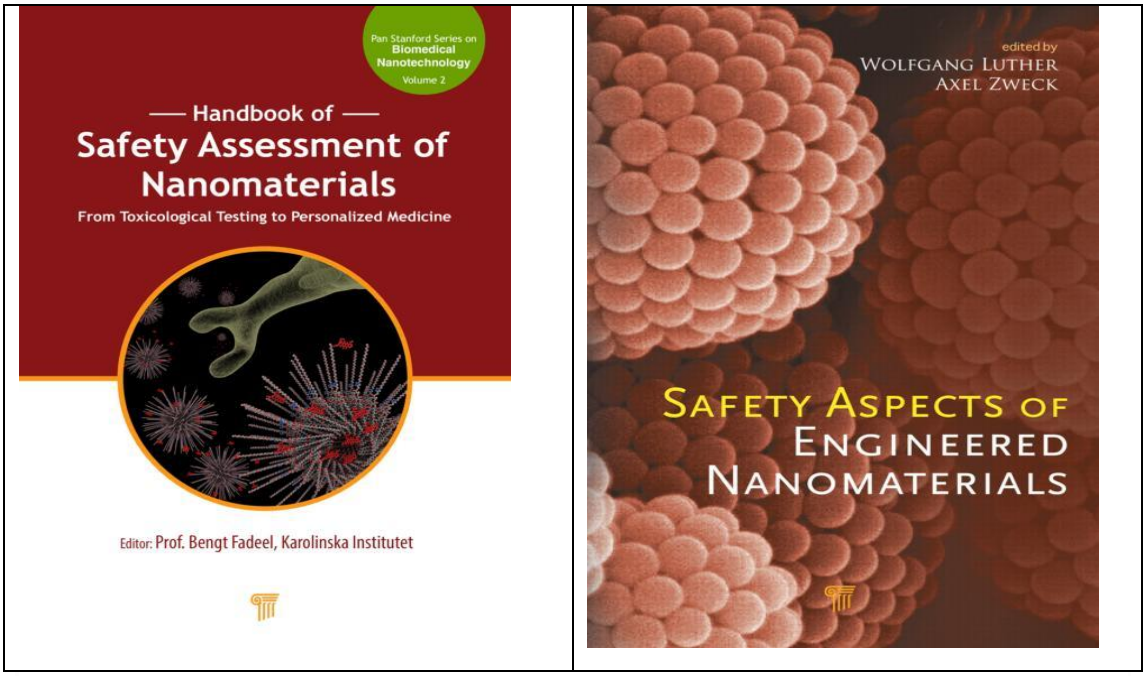

Figure 8. Books on nanomaterials: Fadeel B (Ed), *Handbook of Safety Assessment of Nanomaterials*. CRC Press, Boca Raton, Fl, 2014. Luther W, Zweck A (Eds). *Safety Aspects of Engineered Nanomaterials*. CRC Press, Boca Raton, FL, 2013.

Until now there was a wealth of evidence for the harmful effects of nanoscale combustion-derived particulates (ultrafines particulates of air pollution, $PM_{2.5}$), which when inhaled can cause a number of pulmonary pathologies in mammals and humans. The impact of release of engineered nanoparticles (ENPs) into the aquatic environment is largely an unknown to scientists. A review examined research papers on the uptake of ENPs by endocytotic routes of entry into cells in biological organisms, which may lead to various types of toxic cell injury. Also, the review considered the higher level consequences for damage to animal health, ecological risk and possible food chain risks to humans for inhaled and ingested nanoparticles. Although current toxicity testing protocols should be generally applicable to identify

harmful effects associated with ENPs, research into new methods is required to address the special properties of nanomaterials.[122]

The safety aspects of engineered nanomaterials in the aquatic environment, followed by their ingestion, accumulation and biomagnification in marine organisms in association with adverse health effects, has been the subject of many ecotoxicological studies.[123-128]

Conclusions

Emerging pollutants of concern are increasingly being detected at low levels by new analyticalseparation techniques in municipal waste water, surface water and the marine environment. Environmental scientists are concerned that these compounds and their metabolites are toxic and their presence at higher concentrations may have adverse health effects to humans, aquatic wildlife and sensitive ecosystems. In the last decades there has been a great scientific research effort to study the fate, the occurrence and the ecotoxicology of emerging pollutants in the aquatic environment, especially focusing on their metabolites and transformation products. Emerging pollutants, such as pharmaceuticals (antibiotics, etc), cosmetics, personal care products, perfluorinated compounds, disinfection chemicals, flame retardants, nanomaterials, plasticizers, pesticides, and other commercial products have been the subject of great number of toxicological and ecotoxicological studies and research monitoring projects. Another problem of these emerging chemicals is that released into the environment are subject to processes (biodegradation, photochemical degradation, etc) that changes their environmental behaviour and ecotoxicological profile. This review presents a wide range of research papers and reviews (selected from the worldwide scientific literature) of the last years on the toxicological and ecotoxicological investigations of emerging pollutants.

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