6 Οκτωβρίου 2014

Τοξικές και Επικίνδυνες Χημικές Ουσίες στα Οικοσυστήματα και Επιπτώσεις στα Είδη της ΄Αγριας Φύσης. Επισκόπηση Οικοτοξικολογικών Ερευνών

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Τμήμα Χημείας, Πανεπιστήμιο Αθηνών

Περίληψη: Οι οικοτοξικολογικές έρευνες για τις επιπτώσεις της περιβαλλοντικής ρύπανσης στα είδη πανίδας και χλωρίδας της άγριας φύσης ξεκινούν τον 19^0 και 20^o αιώνα. Αρχικά οι έρευνες εστιάσθηκαν στις γνωστές τοξικές χημικές ουσίες, όπως ο μόλυβδος για τις δηλητηριάσεις πτηνών και των υδρόβιων οργανισμών από τις διαρροές πετρελαίου στην θάλασσα. Η αυξημένη χρήση λιπασμάτων και φυτοφαρμάκων στις δεκαετίες του 1930 και 1940 αποτέλεσαν πεδίο τοξικολογικών ερευνών για τα είδη της άγριας φύσης και ιδιαίτερα των πτηνών στις ανώτερες τροφικές αλυσίδες (σαρκοφάγα και αρπακτικά). Από την δεκαετία του 1960 οι περιβαλλοντικοί τοξικολόγοι διερεύνησαν τις επιπτώσεις μη βιοδιασπώμενων πολυχλωριωμένων διφαινυλίων, διοξινών και άλλων έμμονων τοξικών ενώσεων, που βιοσυσσωρεύθηκαν στους ιστούς ζώων της άγριας φύσης. Την δεκαετία του 1970 οι οικοτοξικολογικές έρευνες ανέπτυξαν πολύπλευρα ερευνητικά πεδία και μαθηματικά μοντέλα, συνδυάζοντας τοξικολογικές μελέτες χημικών ουσιών σε συγκεκριμένους οργανισμούς, οικολογικές αρχές και σύνθετα οικοσυστήματα.

Πολυχλωριωμένες ενώσεις, βαρέα μέταλλα, νέες αγροχημικές ουσίες και φυτοφάρμακα, πολυκυκλικοί αρωματικοί υδρογονάνθρακες, πετρελαιοειδή, βιομηχανικά και αστικά απόβλητα, ρύπανση υδατίνων συστημάτων είναι μερικά από τα θέματα αναδυόμενων ρύπων που έχουν αποτελέσει αντικείμενο περιβαλλοντικών και οικοτοξικολογικών ερευνών τις τελευταίες δεκαετίες. Η επισκόπηση αυτή περιλαμβάνει σημαντικές οικοτοξικολογικές μελέτες και την εκτίμηση οικολογικού κινδύνου σειράς επικίνδυνων χημικών ουσιών που απειλούν τα ευαίσθητα οικοσυστήματα του πλανήτη και έχουν δημοσιευθεί στην επιστημονική βιβλιογραφία.

Πλήρες κείμενο της εργασίας στα αγγλικά [38 σελίδες]: αρχείο PDF, 1,7 MB

<Επιστροφή στη λίστα επιστημονικών θεμάτων και ανακοινώσεων>

6 October 2014

Toxic and Hazardous Chemical Substances in Ecosystems and Adverse Effects on Wildlife Organisms

Review of Ecotoxicological Studies

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Abstract. Wildlife toxicology studies can be traced to the original research efforts of the 19th and early 20th centuries. Research studies focused on well known cases of poisoning evidence of wildlife species, such as lead poisoning by birds and large poisoning of aquatic organisms from maritime petroleum oil spills. The increased use of fertilizers and pesticides in the 1930s and 1940s were investigated by toxicologists in wildlife eagles, and hawks (birds of prey). The discovery in the 1960s of persistent organic pollutants (POPs), PCBs, dioxins and other toxic substances in environmental samples, initiated ecotoxicological studies in wildlife birds and mammals. Ecotoxicology after the 1970s was developed as a multidisciplinary field integrating toxicology of hazardous substances and ecological principles of biological organisms in complex ecosystems.

Polychlorinated compounds, heavy metals, new agrochemicals, pesticides, polycyclic aromatic hydrocarbons and oil pollution, industrial and urban chemical waste, water pollution and other emerging pollutants are some of the new and ongoing research subjects of ecotoxicological studies. Ecological risk assessment of dangerous chemicals are investigated not only at individual species, community or ecosystem level but across multiple ecosystems. Biomonitoring across trophic levels, development of new biomarkers, population modeling, etc, were major research issues in recent years. In this review we present some of the most prominent ecotoxicological studies on wildlife organisms and the ecological risk assessments of ecosystem on Earth that were published in the scientific literature.

1. Introduction: A Historical Perspective of Ecotoxicology Studies

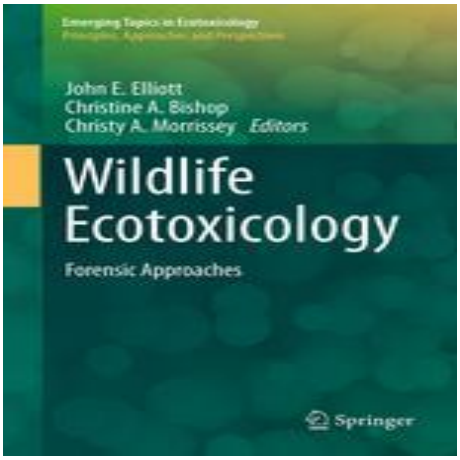
The rapid expansion of chemical manufacturing industry in the 19th century prompted scientists and environmental regulators to investigate the toxicity of raw and industrial chemicals. The principal focus was on human health and occupational exposure, but progressively the focus was directed into environmental pollution and adverse effects on biological organisms an ecosystems.[1] In the early 20th century the first research papers on environmental toxicology appeared in the period 1920-1950. Carpented (1924) published the first series of papers on the effect of trace metals from acid mine drainage on fish in rivers.[2,3] Anderson suggested in 1944 the use of crustacean *Daphnia magna* as a standard toxicity assay organism.[4] Doudoroff and co-workers (1951) advocated the use of standardised fish assays for testing effluent toxicity.[5] Poisoning of birds from ingestion of spent lead shot and predator control agents was another important toxicological study in the 1950s.[6] The threat of toxic petroleum to birds and mammals from exposure to maritime oil spills (classic example the case of Torrey Canyon in 1967) was another interesting toxicological research issue.[7,8]

The synthetic pesticide era with new insecticides in the 1940s and 1950s showed the exponential use of DDT and other organochlorine pesticides. Studies with wildlife birds and small mammals documented acute and chronic toxicity of DDT residues and other polychlorinated insecticides.[9] Biomagnification of polychlorinated insecticides subsequently was found to be occurring worldwide. The term ecotoxicology was coined by René Truhaut in1969. By the late 1960s it became evident that the declining population of some fish-eating raptors (peregrine falcons, bald eagles, and ospreys, etc) was the result of high DDT concentrations observed in their tissues. Studies showed a dramatic decline of the peregrine falcon in Great Britain and North America. The results initiated widespread scientific concern over the possible link between organochlorine pesticide use and adverse ecological effects.[10,11] But the most important clue was the ecotoxicological research on the relationship between DDT contamination and eggshell thickness in peregrines and other breeding birds. The chemical structure of DDT resembles the structure of female reproductive hormones called estrogens that mediate the process of eggshell formation.[12-14]

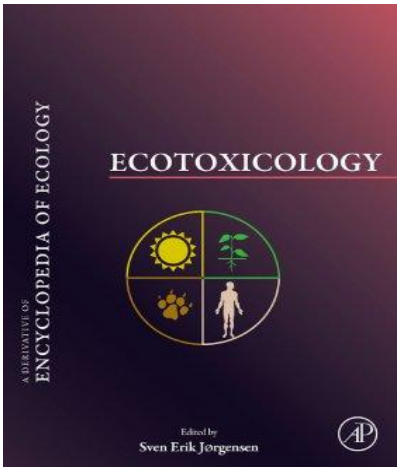
In 1972 U.S. and Europe ban the widespread use of DDT in agriculture. The most significant factor was the extinction of peregrines, bald eagles, and brown pelicans, which were endangered species. Another important factor was the increasing awareness among the public for environmental pollution by industrial and agricultural chemicals and the rise of environmental sensitivities among the population of developed countries. The publication of *Silent Spring* in 1962 by Rachel Carson initiated a public debate on the hazards of chemical pollutants to wildlife.[15,16] By 1963 , a long-term monitoring programme on pollutants and wildlife was initiated in the United Kingdom (UK) and increasing governmental activities on biomonitoring and environmental pollution expanded in the U.S, Canada and in the Scandinavian countries.[17-19]

Figure 1. Wildlife poisoning by pesticides ın the1960s became evident from declining population of some fish and rat-eating birds of prey (peregrine falcons, bald eagles, ospreys, etc). DDT biomagnifications affected their reproduction.

The 1970s was another decade of interesting research investigations for wildlife ecotoxicological problems involving agricultural chemicals. Investigations for anticholinesterase pesticides (inhibition of cholinesterase, use of cholinesterase measurements in surveillance of wildlife poisoning, toxicology of cholinesterase-inhibiting pesticides, and regulatory action).[20] Another study which formulated new ways of research was the wildlife deaths and embryo deformities in snakes and frogs at Kesterson Reservoir (elevated levels of selenium in subsurface agricultural drainwater in Merced County, San Joaquin Valley, California).[21]



- Elliott JE, Bishop CA, Morrissey CA (Eds) Wildlife Ecotoxicology. Forensic Approaches, Emerging Topicsin Ecotoxicology,. Springer Science, Berlin, 2011



Sven Erik Jørgensen (Editor)
Ecotoxicology.
Academic Press, New York, 2010.

Figure 2. Wildlife ecotoxicology and general ecotoxicology textbooks deal with the new developments in ecotoxicology studies.

Ecotoxicological studies on acid precipitation investigated toxicological effects on wildlife forest habitats damaged by acidic rain.[22] Ecotoxicological studies elucidated indirect effects of pesticides operating through the food chain. Secondary metabolites are possible causal factors in the decline of farmland bird species (breeding performance or population survival).[23]

The 1970s showed another advance in ecotoxicology research, especially in the field of ecological risk assessment and modeling. Scientists followed a tiered strategy so that risk assessment can be generated on the basis of a few, simple ecotoxicological tests and worst-case assumptions with regard to exposure. Toxicologists translated responses through modeling. Extrapolating from observation on individuals and a few test species in simple laboratory system to complex natural systems by two methods. First, by dividing endpoints, representing realistic worst cases by fixed extrapolation factors and secondly by using critical response data (EC_{50}, NOEC, etc), as a function of frequency distribution of affected species.[24-26]

In the 1980s studies were expanded to cover hazardous chemicals, such as heavy metal pollution related to mining and smelting, agricultural practices affecting rivers and lakes and their aquatic organisms.[27-30] The **Chernobyl disaster** (Ukraine, 26 April 1986) was the worst nuclear plant accident releasing large quantities of radioactive particles into the atmosphere spreading over much of the western Soviet Union and Europe. Although only 64 people died during the accident itself (firefighters to), estimates of the long-term number of deaths from the accident vary enormously. Radiation covered a large area and 116,000 local people were evacuated.[31]

In 1989 the **Exxon Valdez** accident contributed to a large ecological disaster. A tanker with crude petroleum oil ran into a reef in Alaska's Prince William Sound, 11 million gallons of crude oil spilled into one of the nation's most pristine and productive coastlines. The Exxon Valdez caused the worst oil spill in an area noted for the diversity and abundance of seabirds, marine mammals, fish, and wildlife. The magnitude of the damage to wildlife was a big challenge to ecotoxicologists. The biodegradation of oil and the slow rate of ecosystem recovery were very important lessons to ecotoxicologists.[32,33]

Figure 3. The Chernobyl disaster was the worst nuclear power accident. The Exxon Vandez accident caused extensive damage to a sensitive ecosystem in Alaska Prince William Sound.

New developments in the 1990s were the formulation of studies for evaluation of biochemical biomarker. Biochemical biomarkers have considerable potential for measuring effects of hazardous chemicals under field conditions.[34] For the same decade there is renew interest for chemicals with endocrine disruptive properties and their impact on human health and the environment.[35] Another research area established population modeling in ecotoxicological studies. These studies combined the dynamics of chemically stressed populations as a result of the deduction of population consequences from effect on individuals.[36] Finally, ecological studies for adverse effects of chemical pollutants with amphibians and reptiles were major environmental issues and advances in ecotoxicology. These animals show great susceptibility to chemical contaminants and can be used as sentinel organisms for ecotoxicology studies.[37,38]

Also, in the turn of the century and the beginning of the 21st century there was renew interest in emerging chemical pollutants, such as pharmaceuticals and their metabolites in liquid urban waste, flame retardants and chemical surfactants in various aquatic environments, environmental issues of engineered nanomaterials (ENMs) and pollution by microplastics in aquatic organisms.[39-43]

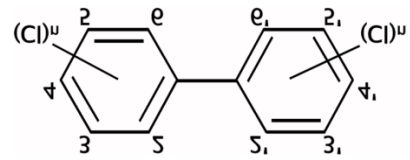
Based upon its history, wildlife toxicology is driven by chemical use and misuse, ecological disasters, and pollution-related events affecting human health or damaging sensitive ecological habitats. Current challenges in ecotoxicology include the need to more thoroughly estimate and predict exposure and effects of chemical-related anthropogenic activities on wildlife and their supporting habitat.

2. Persistent Polychlorinated or Polybrominated Chemicals and Wildlife Ecotoxicology

One group of the most researched hazardous chemicals in the last decades were halogenated, especially polychlorinated, aromatic compounds, typified by the polychlorinated dibenzo-p-dioxins (PCDDs), DDT and Dieldrin, dibenzofurans (PCDFs), biphenyls (PCBs), diphenylethers (PCDEs) and halogenated phenols, anilines and benzenes. These chemicals are highly persistent industrial compounds and agricultural biocides or byproducts of combustion (waste, mineral fuels, etc) which have been widely identified in the environment and in chemical-waste dumpsites. Several studies have confirmed the common receptor-mediated mechanism of action of toxic polychlorinated aromatics in biological organisms. The most toxic dioxin is the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and based on *in vivo* and *in vitro* studies the relative toxicities of individual halogenated aromatics have been determined relative to TCDD (i.e., toxic equivalents, TEs).[44-46]

The high toxicity of these halogenated pollutants increased public and regulatory concern over the potential adverse human health effects and environmental damage to ecosystems. The public perception has been heightened by several incidents in which potentially higher levels of human exposure have been reported. These include agricultural and occupational exposures to chlorinated or brominated aromatics, PCB fires and several accidental exposures (Yusho and Yu Cheng poisoning in Taiwan, the

explosion in Seveso-Italy, the exposures associated with Love Canal in USA, chlorinated phenoxy herbicides with high dioxin levels used in Vietnam as Agent Orange). These incidents, coupled with frequent press reports s, have generated considerable pressure to develop and validate methods for the hazard and risk assessment of toxic halogenated aromatics, with particular emphasis on the PCDDs, PCDFs, and PCBs.[47-52]



PCBs

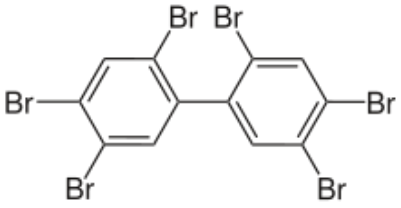
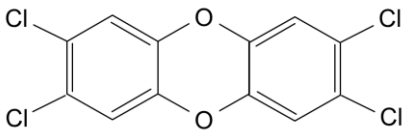
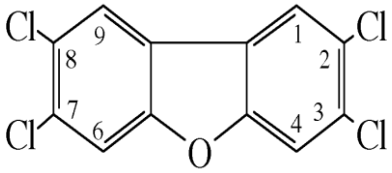


Figure 4. Polychlorinated (PCBs) or polybrominated (PBBs) organic compounds were used in various technological applications but were banned as highly toxic.

Because PCBs and PBBs were implicated in numerous human poisonings in Taiwan and Japan (PCBs) and Michigan (PBBs) from 1985 their use was banned. But their persistence resulted to widely detected in the environment, such as water, fish, wildlife, human adipose tissue, and blood and breast milk. The PCBs and PBBs elicit their toxicological effects through a cytosolic receptor protein which preferentially binds with the pollutants which are approximate isostereomers of 2,3,7,8-TCDD.[53]



2,3,7,8-Tetrachloro-dibezo-dioxin, TCDD, Dioxin



2,3,7,8-Tetrachlotrodibezofurans

Figure 5. Chemical structures of TCDD and tetrachlorodibenzofurans

Ecotoxicological studies in the Netherlands and Belgium were contacted with eggs of the common tern (*Sterna hirundo*) were collected at different locations and incubated artificially. The residual yolk sacs of the hatchlings from the highly polluted colonies, located in the main sedimentation area of the Rhine and Meuse rivers, contained on average 16 ng TEQ (toxicity equivalency) per g lipid (fivefold higher than the concentrations in the reference colony). Average TEQ concentration in chicks that hatched after 23 d of incubation were twice the concentration of those that hatched after 21 days ($p< 0.05$). The non-ortho- and mono-ortho-PCBs were predominant regarding the total TEQ, while the PCDDs and PCDFs contributed less than 10% of toxicity equivalent.[54]

Other studies on acute toxicity of PCBs to birds showed that increasing chlorination of the PCB mixture can lead to mortality. Adverse biological

effects of PCBs include developmental effects, such as endocrine disruption, immunotoxicity and teratogenesis, rather than egg shell thinning (affecting the metabolism of calcium). Dietary no adverse effect concentrations range from 0.5 ppm in the American kestrel to 50 ppm in the Japanese quail. Scientists suggest that genetic differences related to expression of the aryl hydrocarbon (Ah) receptor, rather than toxicokinetics, may be the dominant factor determining reproductive toxicity of PCBs in birds.[55]

Figure 6. Common Tern (*Sterna hirundo*) migratory seabird of the family Sternidae. Breeding in temperate regions of Europe, Asia and North America.

Harbour seals (*Phoca vitulina*) have been used for ecotoxicological studies of persistent organic pollutants (POPs) that have been associated with disease susceptibility and decreased immunity in marine mammals. A study on PCBs, DDT, PBDEs, HCHs and CHLDS (chlordanes) accumulated in the blubber of seals in the central California coast. The highest concentrations of POPs were observed in harbor seal pups that suckled in the wild and then lost mass during the post-weaning fast. The toxicological data in pups and adults of seals illustrate the important influence of life stage, nutritional status, and location on blubber contaminant levels, and the need to consider these factors when interpreting single sample measurements in marine mammals.[56]

Figure 7. Common Harbour seals (*Phoca vitulina*) . The global population of harbour seals is 5–6 million, but subspecies in certain habitats are threatened by marine pollution. Loggerhead sea turtle (*Caretta caretta*) are sentinel species for marine pollution.

Sea turtles are also used by toxicologists for the study of POPs in the marine environment because of their effects on their reproducibility and their sensitivity to toxic effects of POPs. Scientists measured concentrations a large number POPs in loggerhead sea turtle (*Caretta caretta*) egg yolk samples collected from 44 nests in three distinct U.S. locations: North Carolina, Eastern Florida, and Western Florida. POPs concentrations were lowest in W. Florida, intermediate in E. Florida and highest in N. California egg samples, with several statistically significant spatial differences. Scientist suggest that these findings are important to assessing threats among different stocks or subpopulations of this threatened species.[57]

3. Persistent Pesticides and Ecotoxicological Studies

Agricultural pesticides consist of a vast range of synthetic chemicals that kill insects, weeds, fungi, and other organisms to protect the agricultural production and human health. Their use in the last decades have made it possible to feed a growing human population (doubled in 50 years into 7 billions). Also, insecticides protect millions from malaria and other insect-borne diseases, and help make our lives healthier at home and in the countryside. Despite these positive aspects, the potentially serious threats they pose to the environment and the wildlife species have led to a series (in the last decades) of bans on the most dangerous chemicals. In 2013, the European Union took a new step by issuing a partial ban on three neonicotinoids, a widely used group of insecticides suspected of harming bees, butterflies, and other non-target species.[58] Scientists are making strides in precisely understanding the effects of the chemicals, including hundred different ways in which they are broken down in the environment and the harm they cause to wildlife. Some hazardous pesticides have been abolished, alternatives less toxic are introduced. Despite the health and safety instructions and educational projects, 300.000 people (especially Africa, Asia and L. America) commit suicide every year by swallowing intentionally pesticides.[59] Environmental pollution by pesticides and their effects on biota has been the subject of various books.[60,61]

3.1. Pesticides and ecotoxicological studies in birds

Wildlife birds have been from the 1960s, with the case of DDT, the target for ecotoxicological studies in relation to environmental pollution by persistent agricultural pesticides.[62,63]

From a great number of ecotoxicological studies in the scientific literature we select some recent ones for toxic effects of pesticides on birds. Scientist from Spain investigated concentrations and distributions of 57 anthropogenic pollutants, (2009-2012, including 23 organochlorine pesticides, 18 PCBs and 16 PAHs) in liver samples from 102 birds of prey of six species that were found dead or that had died during their stay in the Wildlife Recovery Centers of the Canary Islands. Birds feeding extensively with fish in these areas can represent good bioindicators of environmental pollution in the region. The study found that the species *Accipiter nisus, Falco pelegrinoides*

and *Falco tinnunculus* were the most contaminated. The measured concentrations by DDT and its metabolites, as well as by Dieldrin (polychlorinated), could be considered high in these animals, much higher than reports from other regions of the planet, In contrast, the contamination by PCBs could be considered extremely low. The content of carcinogenic PAHs in these animals was clearly dependent on the feeding pattern of the species; with levels well below the values that were considered toxic in predictive models.[64]

Figure 8. The barbary falcon (*Falco pelegrinoides*).This bird of prey is mainly resident of semi-desert and dry open hills and lays its eggs in cliff-ledge nests. They belong to family Accipitridae, are named goshawks and sparrowhawk

Organophosphorus and carbamade pesticides are widely used in agriculture. In high concentrations can cause acute toxicological; effects in birds. Blood plasma cholinesterase (ChE) activity is a sensitive toxicological biomarker of exposure to these insecticides and several studies have established in the last decade. The *in vitro* sensitivity of cholinesterase from exposure to insecticides such as paraoxon-methyl, carbofuran and carbaryl was investigated in White stork (*Ciconia ciconia*). From the results of toxicological studies it was found that the inhibitory potential of tested anticholinesterase pesticides on plasma of the birds, paraoxon-methyl is the most potent inhibitor followed by carbofuran and finally by carbaryl. Also, the percentage of *in vitro* plasma ChE inhibition was observed to be similar between adults and juveniles.[65] Persistent organochlorine pesticides are used in many areas of the world. In India pesticides residues are identified in liver tissues of various birds. The concentrations were in the range 3.43 µg/g wet wt to 0.02 µg/g wet wt, and the order of accumulation was HCH>DDT>heptachlor epoxide > dieldrin. These concentrations are no serious threat to birds, but continuous monitoring is important.[66]

3.2. Pesticides, trematode infections, and other stressors in amphibian deformities and global decline

Beginning in the mid-1990s, numerous reports of malformed amphibians generated widespread concern among scientists, health officials, and state and federal agencies. Most malformations involved limb deformities, extra limbs, partially and completely missing limbs, skin webbings and bony triangles. Although initial reports mentioned eye abnormalities, internal

irregularities, and tumors, these problems were likely over-emphasized. This issue is still very much alive, and new malformations sites continue to be discovered each year.[67]

Initially, the blame was placed on pesticide residue, but also on infection. Hypotheses proposed to explain the deformities fall into two broad categories: chemical contaminants and trematode infection. Trematode infection (called flukes, or flatworms) occur worldwide causing various clinical infections in humans. The parasites are so named because of their conspicuous suckers, the organs of attachment (*trematos* means "pierced with holes"). Trematode infections such as schistosomiasis have emerged as important tropical infections. Some scientists presented results of field and laboratory experiments that link increased trematode infection, and increased limb deformities, to pesticide exposure. Field experiments conclusively demonstrated that exposure to trematode infection was required for the development of limb deformities in wood frogs.[68]

Figure 9. Field experiments conclusively demonstrated that exposure to trematode parasite infection was the main cause of amphibian deformities.

The idea **that** trematode parasites could cause **malformations** in amphibians was first suggested in 1990 by Dr. Stanley Sessions. Dissection of malformed amphibians revealed an abundance of *Ribeiroia* metacercariae (a group of trematode parasites that infect freshwater snails). He tested the hypothesis by exposing laboratory-raised tadpoles to different numbers of *Ribeiroia* cercariae and allowed them to develop to metamorphosis. Tadpoles exposed to no parasites (control treatment) exhibited high survivorship and no malformations. In contrast, tadpoles exposed to *Ribeiroia* developed deformities and died prematurely.[69] The wordwide decline of amphibians have been attributed to several causes, especially habitat loss (namely “habita split” and disease, ultraviolet radiation and probably some pesticide residues.[70-72] A recent ecotoxicological study investigated the developmental malformations on 3 frog species (*Limnodynastes tasmaniensis*, *Limnodynastes fletcheri* and *Litoria raniformis*) in rice bays of the Coleambally Irrigation Area NSW, Australia. The types and frequencies of abnormalities were typical of reports from agricultural areas with ectrodactyly being the most common aberration. Rice bay surface waters differed significantly in mean pesticide concentrations of atrazine and metolachlor on farms growing rice and corn compared to farms with rice as the sole crop. However, the similar abnormality indices observed in recent metamorph emerging from these two farm types provided

no evidence to suggest a link between larval exposure to the measured pesticides and developmental malformations.[73] Most scientists in the last years suggest that there are multiple causes in amphibian deformities, (inorganic pollutants, parasite infections, predatory invertebrates, UV-B and temperature changes) trematode infection being the most important.[74]

Also, there is an important decline in amphibian populations worldwide. Scientists have reached to the conclusion that there is probably not a single cause for global amphibian declines. A great deal of attention has focused on the role of pathogens in inducing diseases that cause death, but it was suggest that pathogen success is profoundly affected by four other ultimate factors: atmospheric change, environmental pollutants, habitat modification and invasive species. Environmental pollutants arise as likely important factors in amphibian declines because they have realized potential to affect recruitment. Many studies have documented immunosuppressive effects of pesticides and increased pathogen virulence and disease rates.[75] Emerging fungal pathogens pose a greater threat to biodiversity than parasitic groups, causing decline of many taxa, including bats, corals, bees, snakes and amphibians. Wild animals is very difficult to acquire resistance to these pathogens.[76,77]

3.3. Neonicotinoid pesticides and pollinators. Is there a threat?

In October 2006, some beekeepers began reporting losses of 30-90% of their hives in the USA. While colony losses are not unexpected, especially over the winter, this magnitude of losses was unusually high. Honey bees made headlines when a mysterious condition called ***colony collapse disorder (CCD)*** decimated honey bee colonies in parts of the United States.[78] This is not the first time that beekeepers are being faced with unexplained losses. The scientific literature has several mentions of honey bee disappearances—in the 1880s, the 1920s, and the 1960s. The European honey bee, *Apis mellifera*, plays an important role as a pollinator for major agricultural crops, pollinating $15 to $20 billion worth of crops in the United States alone and more than $200 billion worldwide.[79] Commercial production of many specialty crops like almonds and other tree nuts, berries, fruits and vegetables depend on pollination by honey bees.[80]

Scientists and ecotoxicologists started looking in four general categories for the cause/causes of CCD: a). pathogen (fungi, virus, bacteria), b). parasites (mites), c). management stressors (nutrition, migratory stress), d). environmental pollutants (pollen/nectar availability, exposure to pesticides). A survey of honey bee colonies revealed no consistent pattern in pesticide levels between healthy and CCD-affected colonies when pollen bees and beeswax were tested for the presence of 170 pesticides. The most commonly found pesticide in that study was coumaphos, which is used to treat honey bees for *Varroa* mites. At around 2008 the pesticide class neonicotinoids (clothianidin, thiamethoxam, and imidacloprid) has been accused of being the cause of CCD. These neonicotinoids insecticides were developed in the mid-1990s in large part because they showed reduced toxicity to honey bees, compared with previously used organophosphate and

carbamate insecticides. In 2008, Germany revoked the registration of the neonicotinoid clothianidin for use on seed corn after an incident that resulted in the die-off of hundreds of nearby honey bees colonies. Investigation into the incident revealed that the die-off was caused by a combination of factors, including the failure to use a polymer seed coating known as a "sticker": weather conditions that resulted in late planting of corn while nearby canola crops were in bloom, attracting honey bees. Neonicotinoids are an important group of neurotoxic insecticides acting on amphids, leaphoppers and whiteflies at low doses. Although laboratory studies showed some effects, filed studioes with field-realisitc dosages showed no effects on bees.[81]

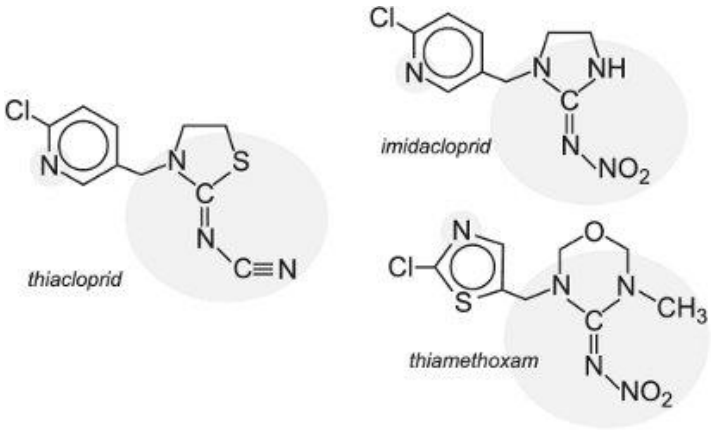


Figure 10. Chemical structures of neonicotinoids

According to the EPA, uncertainties have been identified since their initial registration regarding the potential environmental fate and effects of neonicotinoid pesticides, particularly as they relate to pollinators. Studies conducted in the late 1990s suggest that neonicotinic residues can accumulate in pollen and nectar of treated plants and represent a potential risk to pollinators. There is major concern that neonicotinic pesticides may play a role in recent pollinator declines, are persistent in the environment, and when used as seed treatments, translocate to residues in pollen and nectar of treated plants. The potential for these residues to affect bees and other pollinators remain uncertain. Despite these uncertainties, neonicotinoids are beginning to dominate the market place, putting pollinators at risk. The case of the neonicotinoids exemplifies two critical problems with current registration procedures and risk assessment methods for pesticides: the reliance on industry-funded science that contradicts peer-reviewed studies and the insufficiency of current risk assessment procedures to account for sublethal effects of pesticides. The U.S. Department of Agriculture (USDA) in 2007 established a CCD Steering Committee with representatives from other government agencies, and academia and EPA is an active participant. in the CCD Steering Committee. The Steering Committee has developed the Colony Collapse Disorder Action Plan (PDF).[82]

On the international scale, the Prevention of Colony Losses (COLOSS) research network (more than 300 scientists from 40 countries), was formed to understand and prevent large-scale losses of honeybee colonies and the network published a series of reviews of new research into causes of

honeybee loss, including a special issue of the *Journal of Apiculture Research* in 2010.[83]

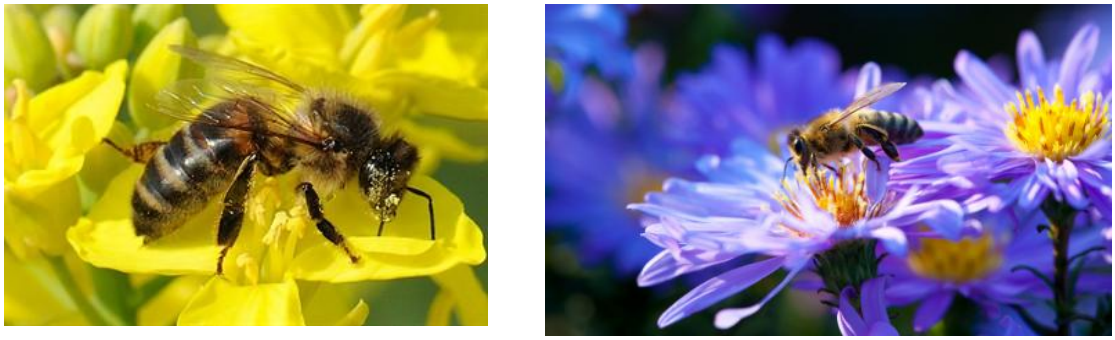


Figure 10. Honey bees (*Apis mellifera,* μέλισσας της μελιτοφόρου,). A combination of mites, viruses, poor nutrition and probably exposure to pesticides caused extensive losses.

In the last years scientists concluded that interactions among multiple factors are the most probable cause of increased colony losses, including the involvement of *Varroa* mites, both alone and in combination with endemic and newly introduced viruses (the microsporidium fungi *Nosema apis* and *Nosema ceranae)*. Also, pesticides and other factors such as forage losses and poor nutrition play a synergistic part. Other reports excluded pesticides as a causal factor but agreed on the concept of multiple factors and the central role of mites and disease.[84] These findings have been echoed by the UNEP, which concluded that the worldwide bee losses are not correlated to the use of pesticides and identified the *Varroa* mite as the primary concern, noting that pathogens and pesticides are also being studied. Using a causal analysis approach, Staveley et al., supported the UNEP conclusion that the major factor in reduced survival of bee colonies is *Varroa*mites; neonicotinoid pesticides were rated as unlikely to be the sole cause but could not be excluded as a contributing factor.[84]

In the European Union The Emerging Risks Unit (EMRISK) was requested by the European Food Safety Authority (EFSA), to set up and coordinate an internal Task Force (TF) to collect, collate and analyse data related to bee risk assessment (for neonicotinoid use), risk mitigation and monitoring. The TF was requested to make an inventory of EFSA's outputs and activities on bees. The TF comprises members of five scientific Units from the Risk Assessment and Scientific Assistance Directorate – Pesticides (PRAS), Animal Health and Welfare (AHAW), Genetically Modified Organisms (GMO), Plant Health (PLH), Scientific Assessment Support (SAS) – as well as members of the Emerging Risks (EMRISK) Unit from the Science Strategy Directorate and members from the Communications Directorate (COMMS).[85]

However, there remains a high level of concern among the public, many regulators, and some scientists, that pesticides may be responsible for weakening honeybees and making them more susceptible to disease, cold, or nutritional stress, or for affecting their learning ability. A comprehensive review of more than 100 publications relating to neonicotinoids and honeybees has been published by Blacquiere et al.[81] Scientific evidence from field studies

showed positive evidence of effects thus obscuring the picture of neonicotinoid effects on bees. Two field studies reported in *Science (2012) put* new emphasis on the problem. Exposure of bubble bees to one neonicotinoid chemical lead to a dramatic loss of queens and could help explain the insects' decline. Whereas exposure of honey bees, to a common insecticide interfered with the foragers' ability to find their way back to the hive. Researchers say these findings are cause for concern and will increase pressure to improve pesticide testing and regulation.[86,87]

But when all the studies are taken together, the reviewers of scientific studies reached the following concluding remarks, “While it is undeniable that overwintering losses of commercial honeybee colonies are higher than they were in the recent past, there is no clear indication that pesticides are the root cause of such losses. The USDA survey shed light on the pattern of honeybee losses across the United States and concluded that such losses were unrelated to the patterns of agricultural pesticide use, in general, or neonicotinoid use, in particular”.[88] Additionally, the epidemiological evidence from Europe shows no correlation of honeybee losses to pesticide use. Parasites and diseases are stronger causal factors other than pesticides, although it is not yet possible to completely discount potential interactive effects of neonicotinoids with other stressors. Also, another indicator is the time of year when increased mortality of honeybees is on the increase. The late fall and over the winter mortality increases, whereas the highest pesticide use occurs in the spring and early summer. The life span of forager bees is very short (approximately 1 month), so the bees that may be exposed to the insecticide in the spring and early summer are not the same bees that overwinter in the hive. Additionally, it has been shown that neonicotinoids do not accumulate over time in the environment, the colony, or the honeybees. Given these two attributes of neonicotinoids and bees, it is not possible for the chemicals to have latent effects that are expressed months after application.[89]

In the last years experts agree that honeybees throughout Europe (and elsewhere) have been severely affected by the introduction of the *Varroa destructor* mite which both parasitizes bees and acts as a vector for a number of debilitating and paralytic honeybee viruses. In addition, honeybee colony losses have increased in frequency across Europe and the USA because of overwintering mortality which is thought to arise from multiple factors, including adverse weather, poor nutrition as well as parasites and disease. Not all parts of the world have experienced recent increases in overwintering colony mortality.[90]

As a precautionary principle the European Commission recently (2014) voted to place a moratorium on the use of three widely used neonicotinoid insecticides (imidacloprid, thiamethoxam and clothianidin) after the European Food Safety Authority (EFSA) determined that there were “high acute risks” to bees from these products through several exposure routes. In their assessment of the risks from imidacloprid on bees, the EFSA considered toxic endpoints for acute (3.7 ng/bee) and chronic exposure (20 µg/kg or L, henceforth ppb) that represent a rough consensus of the toxicity studies reported in the literature. However, they cautioned that there are no guidelines for chronic and sublethal exposure testing in bees, and expressed concern regarding the uncertainty about the biological significance of such

exposures.[91] A vote in the EU (29 April 2013) paved the way for the European Commission to restrict the use of neonicotinoid pesticides containing the three actives: imidacloprid, thiamethoxam and clothianidin. Fifteen countries voted in favour – not enough to form a qualified majority. According to EU rules the Commission will now impose a two-year restriction on neonicotinoids. The UK did not support this – it argued that the science behind the proposal is inconclusive. It was among eight countries that voted against, while four abstained.[92]

4. Heavy Metals in the Aquatic Environment and Ecotoxicological Studies

The rapid development of industry and agriculture in the last decades has resulted in increasing pollution by heavy metals. Significant quantities of heavy metals from industrial and urban areas are discharged into rivers, lakes, coastal areas and seas which can be strongly accumulated and biomagnified along water, sediment, and aquatic food chain, resulting in sublethal effects or death of aquatic populations. Heavy metals in bottom sediments directly or indirectly have the ability for toxic damage to the aquatic flora and fauna. The effects of heavy metal pollutants are detected also on land as a result of their bioaccumulation and bio-concentration in food webs. A high proportion of ecotoxicological studies investigated the toxicological effects of metals on wildlife species and their results of ecological damage in ecosystems.[93-96]

The ecotoxicological methodology for ecological risk assessment has been established in the last decade. The most popular methods used to evaluate the ecological risk posed by heavy metals and other pollutants in ecosystems are: a. the ***index of geoaccumulation*** **(**GEO**) and the *enrichment factor* (**EF**)** risk assessment and b. the ***potential ecological risk index*** PERI*)*.[97] The Potential Ecological Risk Index was proposed by Håkanson (1980) as a contamination control for lakes and coastal systems of Scandinavia.[98]

Biomonitoring of heavy metals has been an appealing tool for risk assessment estimation of metal pollution in the aquatic ecosystems. The bioindicators have been various aquatic species in the trophic pyramid, including algae, macrophyte, zooplankton, insect, bivalve mollusks, gastropod, fish, amphibian, etc.[99] All these species have various advantages and disadvantages in practical biomonitoring of aquatic metal pollution. The common biomonitoring techniques have been classified as bioaccumulation, biochemical alterations, morphological and behavior observation of species, population- and community-level approaches and modeling of adverse health effects through toxicological mechanisms in ecosystems.[100]

Wastewater with metals (Cr, Hg, Ag) and treatment efficiency were evaluated by using the ecotoxicity test with *Chlorella vulgaris (algae)* were performed on them to assess the safety of their environmental discharge. The ecotoxicity was measured with the EC_{50} (effective concentration that causes a 50% inhibition in the algae growth). Ecotoxicity tests can be useful tools for hazardous waste management.[101] Surface sediments were used for the

evaluation of heavy metal pollution in a lake in China. Concentrations and risk assessment of chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) were investigated in surface sediments. Arsenic was found to have the highest acute toxicity by toxic units (TUs), followed by Cr, Ni, Zn, Hg, Cu, Cd and Pb. The potential ecological risk index analysis indicated that As, Cd and Hg had considerable or high ecological risk, whereas Cr, Ni, Cu, Zn and Pb had low ecological risk.[102]

Scientists studying heavy metals pollution are using in feathers of birds of prey (which are in the highest levels of the aquatic food chains) in recent years as bioindicators. Concentrations of Cd, Cu, Pb and Zn show reasonable variation between species, areas and time periods. Feathers of birds of prey have proved to be good indicators of the status of environmental heavy metal pollution. Interpretation of the results requires knowledge on food habit, molting and migration patterns of the species. Several species representing different food chains should be included in comprehensive monitoring surveys. Chick feathers reflect most reliably local pollution conditions.[103]

Most of heavy metals and metalloids are involved in oxidative stress mechanisms in aquatic organisms. Speciation, solubility and complexation are very important factors that influenced toxicity of metals in aquatic species. Fish take up metals through the gills, digestive tract and body surface. Most metals are toxic to enzymes and other biomolecules through free radical mechanisms. Metals that have been investigated for oxidative stress mechanisms and bioindicators in aquatic organisms are Fe, Cu, Cr, Hg, Pb and metalloids As and Se.[104-107]

Mussels are used as sentinel organisms and bioindicators to evaluate the toxic effects of chemical pollutants in marine organisms, especially heavy metals, representing an important tool for biomonitoring environmental pollution in coastal areas.[108-110] Mussels retain metals in their gills and their antioxidant defence enzymes play an important role in cellular antioxidant defence systems and protect from oxidative damage by reactive oxygen species (ROS). Indigenous mussels *Mytilus galloprovincialis* or *M. edulis* have been used for monitoring heavy metal pollution in polluted sites. Seasonal variations of the activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), glutathione peroxidase (GSH-Px) as well as lipid peroxidation (LP) can be used as biomarkers in relation to concentrations of trace metals in their gills, digestive glands and mantle and compared to mussels from an unpolluted sampling sites. These ecotoxicological studies and the “mussel watch” schemes (e,g. Mediterranean Sea) have been proved very effective in long-term monitoring in marine harbors, rivers, lakes, marine and coastal ecosystems.[111-113]

Figure 11. Mussels are sentinel aquatic organisms for studying environmental pollution in aquatic environment and adverse ecological effects.

Metallothioneins (MTs), a family of cysteine-rich, low molecular weight proteins which are localized to the membrane of the Golgi apparatus of living organisms. MTs have the capacity to bind both physiological metals (such as Zn, Cu, Se) and xenobiotic metals (such as Cd, Hg, Ag, As, etc) through the thiol group of its cysteine residues. This mechanism has been proposed to be an important way in the control of oxidative stress and reduction of oxidative damage to proteins, membrane lipids and DNA. Metallothioneins in mussels have been established as bioindicators of metal pollution.[114-116]

5. Hydrocarbons and Polycyclic Aromatic Hydrocarbons (PAHs) and Ecotoxicological Studies

Low levels of petroleum oil and polycyclic aromatic hydrocarbons (PAHs) are naturally present in the aquatic and marine environments. Other anthropogenic sources of PAHs include smelters, the use of fossil fuels (petrogenic) and various methods of waste disposal (such as incineration, pyrogenic origin). Toxicological analysis has provided evidence linking sediment-associated PAHs to induction of phase-I enzymes, development of DNA adducts, and eventually neoplastic lesions in fish. Most studies have focused on high-molecular-weight, carcinogenic PAHs such as benzo[a]pyrene. PAHs are proposed by the U.S. EPA as priority environmental contaminants for aquatic wildlife.[117]

Molecular biomarkers on mussels were used in ecotoxciology studies for the measurement of the impact of oil spills in the marine environment. A well accident was from the “Aegean Sea” oil spill along the Galician Coast, Spain. It occurred on the 3rd of December 1992 when the double-bottom Greek-flagged tanker, “*Aegean Sea*” suffered an accident during extreme weather conditions. The oil spill in Galician coast resulted in extensive ecosystem damage, as well as damage to the fishing and tourist industries. The Galician Coast was an extremely vital source of income for Spain’s fishing industry (crab, lobster, salmon farming, and their main profit came from their production of shellfish). Soon after the oil spill, an ecological study was ordered to evaluate the damage done to the benthic fauna in the region, the

muddy sediments and the ecosystem (determination of species richness, abundance and biomass).[118,119] Mussels *Mytilus Edulis L*. were used to study the effects of oil spill. Whole body aliphatic hydrocarbon concentrations were similar at all sites, but specific chemical ratios indicated a predominance of degraded petrogenic hydrocarbons nearer the oil spill. Concentrations of whole body PAHs (sum of 13 PAHs) increased steadily towards the oil spill, and were paralleled by increases in digestive gland levels of total cytochrome P-450, CYP1A-like protein and lipid peroxidation. No differences between sites were seen for benzo[a]pyrene hydroxylase, glutathione S-transferase, Superoxide dismutase (SOD) and DT-diaphorase activities. Overall the results indicated induction of cytochrome P450(s) and oxidative damage in mussel with oil exposure.[120]

Another major oil spill accident was the *Deepwater Horizon* explosion (British Petroleum, 20.4.2010), which released a US government—estimated 4.9 million barrels of crude oil into the Gulf of Mexico, was responsible for the death of 11 oil workers and, possibly, for an environmental disaster unparalleled in US history. The Macondo well for 87 days continuously released crude oil into the Gulf of Mexico. Many kilometers of shoreline in the northern Gulf of Mexico were affected, including the fragile and ecologically important wetlands of Louisiana's Mississippi River Delta ecosystem. These wetlands are responsible for a third of the nation's fish production and, ironically, help to protect an energy infrastructure that provides a third of the nation's oil and gas supply.[121] Multiple species of pelagic, tidal, and estuarine organisms; sea turtles; marine mammals; and birds were affected, and over 20 million hectares of the Gulf of Mexico were closed to fishing. Several large-scale field efforts and ecological studies were performed, including assessments of shoreline and wildlife oiling and of coastal waters and sediments. Petroleum and the PAH component of oils are known to affect the immune systems of aquatic organisms and wildlife. The effects of oil spill contaminants on immune responses were variable and often exposure dependent, but immunotoxic effects seem to have occured from the DHW spill based on the reported effects of a variety of oils on both aquatic and wildlife species.[122]

Figure 12. Deepwater Horizon oil spill. The Mississipi River Delta ecosystem and many kilometer of shoreline of Northern Gulf of Mexico were affected.

The Deepwater Horizon oil spill was unparalleled among environmental hydrocarbon oil spills, because of the tremendous volume of petroleum oil, the additional contamination by dispersant, and the oceanic depth at which this release occurred. Analysis of water collected in the area indicated that samples (20%) were toxic to bacteria based on the Microtox assay, 34% were toxic to phytoplankton via the QwikLite assay, and 43% showed DNA damaging activity using the λ-Microscreen Prophage induction assay. The Microtox and Microscreen assays indicated that the degree of toxicity was correlated to total petroleum hydrocarbon concentration. Long-term monitoring of stations showed that Microtox toxicity was nearly totally absent by December 2010 (8 months after the accident) in the Northeastern Gulf of Mexico. In contrast, QwikLite toxicity assay yielded positives at multiple stations or depths, indicating the greater sensitivity of the QwikLite assay to environmental factors. The Microscreen mutagenicity assays indicated that certain water column samples were mutagenic at least 1.5 years after capping the Macondo well. Similarly, sediment porewater samples were also highly genotoxic.[123]

The Deewater Horizon oil spill affected coastal estuaries in the area. Coastal estuaries are among the most biologically productive habitats on earth. The 2010 Deepwater Horizon oil spill contaminated hundreds of kilometers of coastal habitat in the Louisiana's Delta. Exposures to petroleum oil and its toxic components, in addition to their direct toxic effects, oil may interfere with functions that normally enable physiological compensation for suboptimal conditions (salinity, hypoxia, pathogens, and competition). These interactions pose challenges for accurate and realistic assessment of risks. Ecotoxicologists must adopt integrative and holistic measures of effects in order to characterize and solve ecotoxicological issues in the wildlife.[124]

Seabirds, as top marine predators, have been proposed as suitable biomonitors for pollutants such as PCBs or heavy metals, but in the case of PAHs, the suitability of seabirds to serve as biomonitors remains unclear. The yellow-legged gull (*Larus michahellis*) was proposed as a good indicator of PAH contamination after comparing levels from colonies polluted by the Prestige oil spill with non-affected areas.[125] However, whereas some seabird studies have found relationships between PAH levels and trophic or geographic variables, other studies have not. Studies of PAHs in seabird eggs have documented low concentrations for certain geographic areas and species, suggesting neither biogeographical trends nor interspecific patterns for PAH levels.[126] Some authors have reported that PAHs are not commonly found in the tissues of birds from non-contaminated sites, and when PAHs are found, they tend to be present at low levels. Studies also found greater PAH burdens in birds feeding on invertebrates (low trophic positions) compared to those feeding on vertebrates, such as fish (higher trophic positions).[127]

In a recent study (2011) the PAHs (proposed by the U.S. EPA as priority environmental contaminants) were analyzed in the livers of pelagic seabirds (*O. Procellariiformes*). Scientists used a multi-species approach, including petrel and shearwater species with different trophic niches and breeding separately in the Atlantic and the Mediterranean to investigate whether PAHs in seabirds result from dietary differences or spatial differences in PAH exposure. The liver was selected as the target tissue to measure PAH

exposure associated with the breeding areas of the seabirds. Birds rapidly metabolize and readily excrete PAHs. PAHs were analyzed in the liver of 5 species of pelagic seabirds (Procellariiformes) from the northeast Atlantic and the Mediterranean. Measurements observed that there is not significant spatial patterns, only minor effects of the geographic origin on seabird PAHs. The study found significant higher PAH levels in petrel compared to shearwater species, which could be related to differences in their exploitation of mesopelagic and epipelagic resources. Overall, seabirds emerged as poor indicators of pelagic chronic PAH levels.[128]

Figure 13. Procellariiformes is an order of seabirds that comprises four families: the albatrosses, petrels and shearwaters, storm petrels, and diving petrels.

6. Emerging Pollutants and Contaminants and Ecotoxicological Studies

In the last decades there were rapid technological and research developments that resulted in the invention and production of a great variety of new materials with large number of practical applications in industry, transport, health, communication and everyday life of humans. As a result of massive use of these new products emerging pollutants or contaminants appeared (initially at low concentrations) in water systems, soil, river, lake and marine sediments and air. Most of these materials are persistent, with low biodegradation rates and the potential to bioaccumulate in tissues of biological species and in humans. These materials have been called “emerging contaminants or pollutants” because they were detected by new and highly sensitive analytical methods in environmental samples. Although their toxicological and ecotoxicological properties are not well established yet, they are perceived as potentially dangerous to the environment and human health.[129, 130]

a. **Plastic materials** having a variety of useful and flexible properties and finding myriad of applications. Plastic waste and microplastics are perceived as very dangerous for the marine environment and the marine biota.

b. New pharmaceuticals, antibiotics, disinfectants and personal care products. Their increased production in thousands of tones every year and

their presence in municipal wastewater and drinking water are a cause of emerging concern among scientists

c. Nanomaterials with a great variety of properties and hundreds of useful applications in many widespread products. New analytical techniques at substantial low concentrations have detected recently nanomaterials in many environmental samples. The presence of nanomaterials in the aquatic environment and their small size

d. New persistent chemicals (additives, surfactants, perfluorinated compounds, flame retardants, etc) with a great spectrum of practical applications. Some of these chemicals have endocrine disrupting properties and other toxic effects.

There is mounting scientific evidence that even at low levels some of these emerging pollutants in the environment may affect wildlife, sometimes causing non-lethal effects but under chronic exposure adverse ecological effects in sensitive ecosystems.[131,132]

All these subjects of emerging pollutants, especially in the aquatic environment, have been published recently.

a Valavanidis A, Vlachogianni Th. Microplastics in the marine environment. Ubiquitous and persistent pollution problem in the world oceans threatening marine biota. WEB site www.chem.uoa.gr, Research Studies (Επιστημονικά Θέματα και Ανακοινώσεις) 26.6,2014 (34 pp).

b. Vlachogianni Th, Loridas S, Fiotakis K, Valavanidis A. Engineered Nanomaterials and Nanotechnology Applications. Health and Safety Problems from Exposure to Nanoparticles and Nanomaterials and Their Impact in Environmental Pollution. WEB site www.chem.uoa.gr, Research Studies, March 2014, (33 pp).

c. Vlachogianni Th, Valavanidis A. Nanomaterials: environmental pollution, ecological risks and adverse health effects. Nano Science and Nano Tecnology (review). An Indian Journal. 8(6):208=226, 2014.

d. Vlachogianni Th, Fiotakis K, Loridas S, Valavanidis A. Environmental Pollution by Endocrine Disrupting Chemicals. Adverse developmental reproductive and immune effects in the wildlife and in human health. WEB site www.chem.uoagr, Research Studies 3.6.2013 (54 pp).

e. Vlachogianni Th, Valavanidis A. Chemical pollutants with endocrine disrupting properties: Adverse health effects to humans and wildlife. WEB site www.chem-tox-ecotox.org, 17.9. 2013 (55 pp).

f. Vlachogianni Th, Valavanidis A. Pharmaceuticals and personal care products as contaminants in the aquatic environment. A category of organic wastewater pollutants with special characteristics. *Pharmakeftiki* 25(1):16-23, 2013.

7. Current Developments: Bioassays, Biomarkers, Community Ecotoxicology, Toxicogenomics in Wildlife Species and Ecosystems

Environmental toxicology and ecotoxicological studies and research have gone through big challenges and changes in the last decades. The biggest challenge was to develop and apply biotests that can target different levels of biological organization, from biomolecules and cells, to tissues and

organs of individual species, to populations, communities, trophic levels and ecosystems.[129]

Bioassays (biotests) must be able to perform under standardized conditions, depending on the end result of the measurement. It can be a single-cell system (laboratory experiments *in vitro*), a test biorganism, animal or plant (laboratory experiments *in vivo*), using a simple community of biological organisms under laboratory conditions (experiments are called microcosms or mesocosms) and in filed study with ecological significance (with *in situ* exposure) of a functioning ecosystem, e.g. lake, river, with all its living organisms and abiotic conditions. Also, ecotoxicologist can use in their studies sensitive molecular biomarkers (cellular and/or physiological alterations), as well as histo-pathological evaluations on organisms. Finally, community indices (which represent ecosystem integrity) can be used in ecotoxicological studies to measure tolerance to pollutants over time.

The *in vitro assays* allow the rapid and sensitive detection of toxic chemical activity in biological systems. These test measure effects at the cellular level and are designed for applications in the laboratory. In vitro tests can be used for the screening of large number of environmental samples for the identification of pollution “hot spots”.

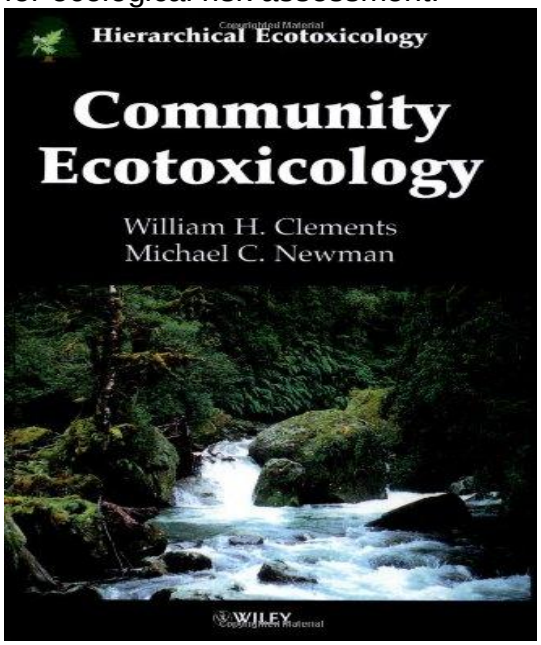
The *in vivo bioassays* can be performed in single species or multiple species. In these biotests whole living organisms (model species) are exposed to ambient samples (water, sediment, extracts) or increased concentrations at laboratory conditions and in short period of time measurements are performed for their responses (biochemical, enzymatic, behavioural, acute lethal, chronic, etc). These experiments allow the quantification of toxic effects. The bioassays’ protocols are set and have to meet strict quality standards by the Organization for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO) Other important bodies for validation and standardization of bioassays/toxicity tests are the American Society for Testing and Materials (ASTM) and the US Environmental Protection Agency (EPA). In Germany the DIN Institute Deutsches Institut fur Normung (German Institute for Standardization).[130]

To text the toxicity of individual chemicals and their environmental risk assessment is the combination of hazard and exposure evaluation. First determination of toxicological effects on organisms and then quantitative analysis of adverse effects at different exposure concentrations. Of critical importance is the determination of effect concentration/dose EC_{50} or ED_{50} or the endpoint of lethal concentration/dose LC_{50} or LD_{50} and the NOEL (no observed effect concentration).[131]

Multispecies bioassays advance one step further. The biotests of single organism have several limitations and in order to increase the environmental and ecotoxicological significance the multispecies test were used, mimicking natural communities or food webs. Similtaneous exposure of multiple species integrate interactions among species (competition and predation). Measurements of exposure or effect indicators in field studies under natural environmental conditions have additional advantages. Organisms are exposed *in situ* and therefore their biomarkers have more ecological significance than laboratory tests. Especially for wildlife species the

field studies are very important. Field studies and methods for *in situ* measurements are very diverse and in general are not standardized. They include caged studies (animals are held inside cages which are inserted in the aquatic environment for a certain period), bypass (flow-through) systems in which polluted water flow through special vessels, and transplanted organism studies (like mussels in nets). These studies are conducted with fish and/or macrtoinvertabrates. The *in situ* studies integrate the combined effects of complex environmental conditions and potentially variable exposure to toxic chemicals, facilitating the assessment of ecotoxicity in the field.[132-138]

Biochemical Biomarkers in ecotoxicology proved to be important analytical tools for biomonitoring in ecotoxicology studies. They can measure sublethal exposure of organisms to toxic and dangerous environmental chemicals or stressors in general. Biomarkers are sensitive indicators, specific, simple to use and suitable for bioassays for acute and chronic exposures. For example the measurement of inhibition of serum 'B' esterases to monitor exposure of birds to organophosphorus insecticides, the measurement of DNA damage caused by aromatic hydrocarbons, the measurement of antioxidant enzymes under the oxidative stress caused my metals, et c.[135] Biomarkers are analyzed directly in cells and tissues of exposed organisms and are traditionally subdivided into molecular, biochemical, cellular and physiological alterations caused by external pollutants and stressors. Biomarkers can provide early warning signals of declining organism health and physiology changes under increasing environmental pollution. Biomarkers can be misused but following appropriate standards and sensitive biochemical procedures can provide valuable results for ecological risk assessment.[139-141]



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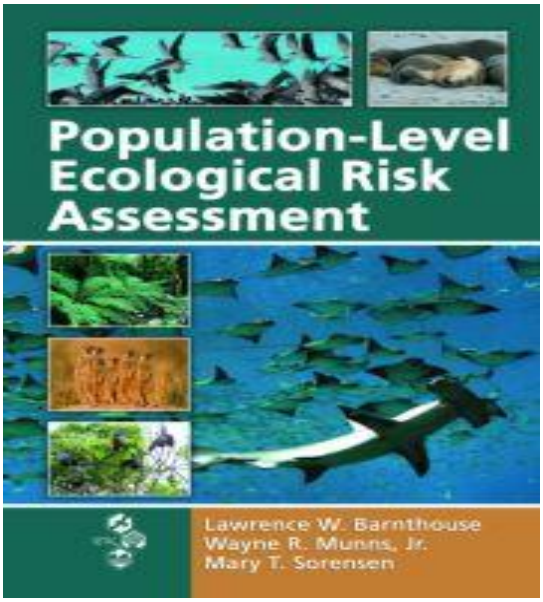

Barnthouse LW, Munns WR, Sorensen MT. *Population-Level Ecological Risk Assessment.* CRC Press, Boca Raton, FL, 2007

Figure 14. Books on Community ecotoxicology and population-level ecological risk assessment. Community indices have high ecological relevance.

Ecological effects of pollutants/contaminants may occur at several levels of biological organization. Organism communities consist of interacting populations that overlap in time and space. Thus, **community ecotoxicology** is concerned with effects of pollutant/contaminants on communities. Community indices have high ecological relevance. Community responses can be described in terms of changes in structure (number, abundance, diversity of species) and function or tolerance to pollutants over time. Although community indices have limited ability to identify causes and stressors, such as organic pollution, eutrophication, exposure to toxic chemicals or habitat degradation by environmental pollution they are important for the study of ecotoxicological problems of ecosystems and evaluation of ecological risk assessment.[142]

Ecotoxicogenomics. In the last decade there was a rapid progress in the field of genomics (the study of how an individual's entire genetic make-up, the genome, translates into biological functions). Genomics provide tools that may assist our understanding of how chemicals can impact on human and ecosystem health. The challenge in ecotoxicology for the 21st century was to understand the mechanisms of toxicity to different wildlife species. Scientists used the term 'ecotoxicogenomics' to describe the integration of genomics (transcriptomics, proteomics and metabolomics) into ecotoxicology. Ecotoxicogenomics is defined as the study of gene and protein expression in non-target organisms that is important in responses to environmental toxicant exposures.[143]

Genomic approaches, or “omics” as they are currently referred to, encompass transcriptomics (mRNA), proteomics (proteins), and metabolomics (metabolic signatures from resulting activity), and incorporate epigenetics (heritable changes in expression), and genotyping (DNA). The proliferation of different genomic was a combination of advances in biological, instrumental and bioinformatic techniques,[144,145]

a. **Transcriptomics**, otherwise known as “global analysis of gene expression”, examines the genes and corresponding biochemical pathways that are involved in various biological processes. These techniques are highly sensitive indicators of an organisms' interaction with their environment.

b. **Proteomics** represents the high-throughput assessment of the functional responses of gene expression; the proteins and peptides, as well as protein-protein interactions. Proteomics combined with bioinformatic analyses, can be used to assess functional biochemical pathways that respond to environmental and/or contaminant/pollutant stimuli. Proteomics can offer a more robust approach for ecotoxicological risk assessments.[146-148]

c. **Metabolomics**, genomic approach measuring the concentrations of metabolites that represent enzymatic activity upon xenobiotics, and associates these, through bioinformatic techniques, with changes in biological functions in the exposed organism.[149,150]

d. **Epigenetics** is another emerging field of genomic approach that is rapidly being incorporated into ecotoxicological studies. It investigates the alterations in gene function or cell phenotype, without changes in DNA sequences. Epigenetics can play a role in interactions between

chemicals and exposed species, between species and abiotic ecosystem components or between species of the same or another population in a community. Epigenetic changes in plants, insects and cladocerans have been reported to be induced by various environmental pollutants or stress factors. In the case of hazardous chemicals, studies in rats and mice exposed to specific pesticides, hydrocarbons, dioxins, and endocrine disrupting chemicals demonstrated the induction of epigenetic changes, suggesting the need for further research with these substances in an ecotoxicological context.[151-154]

e. **Genotyping** is another genomic approach incorporated into ecotoxicological assessments. It is potentially genotyping techniques that will assist comparative species toxicology, and help understand what sensitivity means from one organism to another. This is an especially important parameter that must be considered within the context of ecosystem resilience.[155]

Ecosystem assessment and ecosystem services. The next step in the ecotoxicological studies is to link effects of pollutants from the biological level to ecosystem health. In this respect toxicologists need an integrated and multifaceted approach based on existing knowledge. They have to select biological indicators, bioassays of standard whole organisms, biomarkers and *in vitro* tests and to combine chemical pollution to ecosystems measurable toxic effects. The greatest challenge in ecotoxicology is the linking of chemical exposure levels to ecosystem functioning and **ecosystem services**. Ecosystem adverse effects comprise all abiotic and biotic changes that exceed the natural; change rate or frequency. While the concept of ecosystem services relies on all values and functions that ecosystems provide to the natural environment and the mankind. These services include provision of food, clean drinking water, recreation, aesthetic and ethical values. The ecosystem services concept was popularized by the **Millennium Ecosystem Assessment** (MA) in 2000.[156,157]

The Millennium Ecosystem Assessment (MA) was called for by the United Nations Secretary-General Kofi Annan in 2000 and initiated in 2001. The objective of the MA was to assess the consequences of ecosystem change for human well-being and the scientific basis for action needed to enhance the conservation and sustainable use of those systems and their contribution to human well-being. The MA has involved the work of more than 1,360 experts worldwide. Their findings, contained in five technical volumes and six synthesis reports, provide a state-of-the-art scientific appraisal of the condition and trends in the world's ecosystems and the services they provide (such as clean water, food, forest products, flood control, and natural resources). Ecosystem services were grouped into four broad categories: i. provisioning (the production of food and water); ii. Regulating (control of climate and disease); iii. Supporting (nutrient cycles and crop pollination; and iv. Cultural (spiritual and recreational benefits).[158-160]

The institutions involved in MA were: UNEP provided overall coordination for the MA (administration, director and more than half of the core financial support). The MA Director's office was based in Malaysia at the WorldFish Center, as was the TSU for the Sub-Global Working Group.

UNEP's World Conservation Monitoring Center (UNEP-WCMC) hosted the TSU for the Condition and Trends Working Group, and the International Council for Science's (ICSU) Scientific Committee on Problems of the Environment (SCOPE) supported the Scenarios Working Group. The Institute of Economic Growth in Delhi supported the Responses Working Group. The World Resources Institute, in partnership with the Meridian Institute, supported the MA's outreach and engagement activities, and coordinated the publications process [http://www.unep.org/maweb/en/About.aspx#].

Conclusions

Environmental sciences and relevant research developed rapidly since the 1960s responding to the alarming effects of pollutants to the aquatic environment, to human health, wildlife and ecosystems. Much has been achieved in the last decades in many developed industrial countries in the fields of emission reductions, legislation, nature restoration, conservation of wildlife species, and ecological risk assessment of dangerous chemicals and other stress factors. A great number of wildlife species have recovered in many parts of the world from the initial decline observed by scientists. Research projects on wildlife ecotoxicological studies have increased substantially and new methodologies applied for better risk assessment. Although there are difficulties in determining causality due to poor understanding of natural variability of population dynamics, the multiple factors influencing sensitive ecosystems and the synergistic effects, wildlife ecotoxicology has made substantial progress. Despite the complexity of laboratory bioassays with wildlife species, the influence of confounding factors in the field studies, wildlife developed new modeling and ecotoxicological observations for proper ecological risk assessment. This review explored the historical background of wildlife ecotoxicology and the dimensions of the various disciplines developed throughout the years. The following sections described some important ecotoxicological fields with hazardous chemicals and ongoing research projects in emerging ecological problems. Finally, the review covered current development in toxicology and research, new bioassays, biomarkers, community ecotoxicology and toxicogenomics. The sections of the review are covered by numerous references from the scientific literature and recent research paper on the ecotoxicological subjects.

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