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Utilization of Marine Crustaceans as Study Models: A New Approach in Marine Ecotoxicology for European (REACH) Regulation

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1. Introduction

During last decades the productive activities, the increasing energy demand and the massive resource exploitation have caused extensive pollution phenomena that are to date spread on a worldwide scale. Therefore, the monitoring and assessment of environmental pollution is a subject of high concern owing to the implications that pollutants can exert on the environment, organisms and ecosystems, as well as on the quality of life of humans and on public health.

Pollution problems affect greatly the aquatic environments that are mainly sensitive to several typologies of contamination, such as chemical pollution, oil dumping, microbiological contamination from sewers, etc. These inputs can exert devastating effects on ecosystems with long-term consequences (Mille et al., 1998).

To date a lot of chemicals are utilized in productive processes and many new substances are synthesized every year; the utilization and introduction of these newly synthesized chemicals into the environment and in production cycles must be approved after an accurate evaluation of their eventual toxic properties against selected organisms with the main purpose to protect the safety of plants and animals and the human health. To do this several experiments useful to test the effects consequent to contact, inhalation and ingestion and to estimate the risks connected to the acute/chronic exposition in the natural and work environments have been proposed with the aim to define some fundamental parameters, such as the acceptable dose, the risk dose, the lethal dose, etc.

These evaluations need to be carried out using test-species which are representative of the environmental compartment under consideration; in this connection, the availability of test-species able to furnish reliable and cheap results and to evaluate the activity of pollutants at the individual and ecosystem level is essential. Nevertheless, it is known that the tests on animals have ethical implications and often show problems connected to the reliability and to the application of results to the natural conditions. As a matter of fact, the test-organisms have their own physical, biological and biochemical characteristics and thus they can metabolize some substances, and suffer their effects, in a different way from each other and, in particular, differently from humans.

To date the availability of test-species, easy to collect and to rear, and sensitive to different xenobiotics, is an important aspect in ecotoxicology in order to characterize the risk of chemicals. In general, in toxicity tests some organisms belonging to a target-species are exposed, in controlled conditions, to the activity of the samples to be investigated (water, sediments, soil, sewage, sludge, chemicals, known toxicants, etc) in order to evaluate the eventual toxic effects. At the end of tests lethality or sub-lethality can be observed according to the considered end-point (mortality, growth, motility, physiological and reproductive alterations, etc.) and as a consequence of the utilized species and of the extent of measurable effect; furthermore, acute or chronic toxicity can be distinguished according to the duration of the test compared with the life cycle of the organism.

It is well known that different species have different ecological and biological characteristics; for this reason, to achieve an adequate description of the environmental injury using a single species is not possible in the laboratory. For this reason, the preparation of batteries of tests including some different species is a suitable procedure; selected species should be used in the tests on the basis of criteria useful to satisfy most of the requirements to correctly perform the ecotoxicological assessment.

Overall, the criteria useful to choose different test-species should comprise: the different phylogenetical position and trophic level, the different ecological relevance, the sensitivity to specific contaminants, the relative shortness of the life cycle, the easy availability, the known adaptation to laboratory conditions, the possibility to respond to the different ways of exposition to contaminants. Furthermore, the main requirements of a toxicological test can be summarized as: standardization, possibility to give replicates, easy realization, possibility to discriminate between different results, cost reduction, rapidity of execution (Onorati & Volpi Ghirardini, 2001).

In the aquatic environment an ideal battery of organisms should comprise the representative links of the food web: a primary producer, such as a microalga, a primary consumer (invertebrate), such as a crustacean, and a secondary consumer (vertebrate), such as a fish (Shaw & Chadwick, 1995), taking into account the specific application, the typology of the considered environment, the presumptive levels of pollutants, the physico-chemical characteristics of the involved substances, the purpose of the ecotoxicological study, as well as the available resources.

In this connection, the new European regulation REACH (Registration, Evaluation, Authorization of Chemicals) No. 1907/2006 introduces an integrated system for the management of all produced/imported chemicals for an amount ≥1 ton/year and states that all substances destined to be used in the EU and to be introduced into the production processes must be subject to accurate evaluation including toxicity tests on selected organisms.

All tests indicated by REACH must be carried out in conformity with well defined analysis methods determined by the EU or, failing that, according to the OECD guidelines or to other determined methods. Furthermore, all tests must be performed in conformity with the principles of Good Laboratory Practice (GLP) according to the pertinent Community directive.

2. A global view on reach regulation

The REACH regulation supplies information concerning what test must be performed to evaluate chemicals in different situations.

Acute toxicity tests concern the evaluation of adverse effects which can be observed after a short-term exposition (hours or days according to the utilized species); these tests should be carried out applying the OECD guidelines.

The repeated exposition can be distinguished in I) the sub-acute (or sub-chronic) one that concerns the studies with a daily exposition to chemicals of longer duration than acute ones, but not exceeding a defined part of the life span of the organism; for example, for fish species it must not exceed a period equivalent to one-third of the time taken to reach sexual maturity (Solbé, 1998) and II) the chronic one that concerns an exposition extending for all or for most of organism life span. The adverse effects of expositions concern the alterations of morphology, physiology, growth, development, reproduction and survival.

The reproductive toxicity concerns the effects on reproduction and fertility in adults and the development toxicity studies the effects on offspring. These tests are characterized by multiple endpoints which consider the reproductive disability or harmful non-hereditary effects on offspring. The REACH regulation provides for a screening test, a prenatal toxicity test (on one or two species) and a reproduction toxicity test for two generations. These tests should be carried out according to OECD methods.

Mutagenic, clastogenic and carcinogenic effects with permanent and transmissible changes of genetic material, structural chromosome aberration, change of chromosome number and genotoxic effects concern processes able to change the structure of DNA and the genetic information.

Degradation/biodegradation and bioconcentration/bioaccumulation are also considered in REACH regulation, with the advice to use OECD or other alternative methods. These studies are carried out on aquatic organisms as well as, in some situations, also on soil organisms such as earthworms and seeds.

2.1 Aquatic toxicity

The aquatic toxicity of chemicals is one of main aspects of REACH regulation and an important parameter for the evaluation of substances. As a matter of fact, water is the principal constituent of all living beings and in most of them it constitutes more than 70% of wet-weight. A lot of energy transfers, substance diffusion and enzyme reactions take place in waters; for this reason it has a pre-eminent biological concern. Therefore, the evaluation of the ecotoxicity on aquatic organisms is a fundamental step in the whole evaluation process of a chemical.

In ecotoxicity testing the organisms are exposed to different concentrations of chemicals/contaminants that can be assumed through respiration or teguments; then the balance repartition mechanisms between water and absorption compartments take place with the result of a progressive increase of the toxicant into the body (bioconcentration). After absorption the toxicant is subject to the distribution and to metabolic processes as well as to excretion; for these reasons it is difficult to estimate the internal concentration of toxicants and conventionally the toxicity is quantified in terms of concentration of the substance in the medium (Gaggi, 1998; Paoletti et al., 1998). In particular in aquatic ecotoxicology, and mainly when the invertebrates are considered, it is very difficult to estimate the amount of toxicant assumed into the body; so, this parameter is unknown, but is known the concentration of the toxicant in the water. Anyhow, it should also be considered that to vertebrates (fish or mammals) the toxicant can be administered directly into the body (blood and/or muscle) and therefore its amount is certainly known. So, these two cases are remarkably different and in the first case we can express the results as LC_{50} (Lethal Concentration) while in the second one as LD_{50} (Lethal Dose).

The tests can be subdivided in acute and chronic. The acute toxicity concerns experiments carried out for hours or days and is generally expressed as LC_{50}, that corresponds to the concentration able to reduce the survival of exposed organisms up to 50%, or EC_{50}, that corresponds to the highlighting of an adverse measurable effect such as immobilization. The chronic toxicity regards a long-term exposition (weeks, months) and theoretically can be extended during the whole life cycle of the organisms; the current endpoints are the NOEC (No Observed Effect Concentration) and the LOEC (Lowest Observed Effect Concentration) that generally consider the survival, growth and reproduction. The regulation recommends to use standardized methods but also well described non-standardized protocols or modified methods can be acceptable.

2.2 Test-organisms in aquatic toxicity

In aquatic toxicity tests procariotic organisms, algae, plants and animals having particular characteristics are used as test-species. In general, a test-species must show a known sensitivity to a stress agent, so in the presence of this agent it will suffer alterations of life functions, growth inhibition, reproductive and metabolic disorders or, on the contrary, it can find favourable conditions and develop to the prejudice of other species. It follows that to elect a species to the role of test-species is not easy because each species has its own sensitivity and therefore furnishes a different response (Calamari et al., 1980).

A fundamental factor is the "basic" knowledge of the test-species, that implies the knowledge of life cycle, natural mortality rate of the population and mortality rate of the first stages in order to avoid interferences with the mortality due to the toxic stress. As concerns the response it is necessary to consider that generally species that can survive and reproduce in various environmental conditions are more tolerant to toxicants than species adapted to live in defined conditions.

The research concerning the employment of animal organisms in ecotoxicology have had a remarkable impulse during the last two decades and several species have been used in ecotoxicological tests; so, the list of species that have been proposed to have a role in ecotoxicology is very long and is still in progress.

To date in ecotoxicology the principle that the potential toxicity of a substance can be evaluated only with batteries of ecotoxicity tests is accepted. Each battery must have at least three test-species with well defined life-stages; overall, the test should be carried out considering the different levels of the food web; therefore, it is essential to use a primary producer, such as an unicellular alga, a primary consumer, such as a filter feeder invertebrate, and a secondary consumer, such as larval fish. Also a saprotroph/saprophyte and a detritus-feeder should be comprised among the considered species (Baudo et al., 2011). Useful results could be also obtained through *in vitro* systems, such as cell cultures of fish cells (Pane & Mariottini, 2009). Finally, the test battery should have a good sensitivity and a discriminating potentiality in order to respond as much as possible to pollutants (Baudo et al., 2011).

Among aquatic organisms crustaceans have a key-role in the environment for their intermediate position in the food web and also for their wide distribution and high density; for this reason in ecotoxicological testing several crustacean species have been proposed (APHA, AWWA, WEF, 1995) and are having a wide employment both in freshwater and in marine ecotoxicology.

3. Utilization of crustaceans in ecotoxicology

Small crustaceans are an important link within the food web, playing an important role as primary consumers and sometimes also as secondary consumers, so they are eligible to be used in ecotoxicological evaluations; as a matter of fact, they connect the energetic fluxes between the primary producers (mainly algae) and the consumers of higher levels (such as fishes) and, therefore, they are placed at a key-level into the food web. To date only the freshwater cladoceran *Daphnia magna* is approved as suitable crustacean for aquatic tests in freshwater ecotoxicology.

3.1 Freshwater crustaceans

Daphnia magna is the most important test-species in freshwater ecotoxicology (Persoone & Janssen, 1998). The parthenogenetical reproduction in *Daphnia* allows to have identical specimens useful for testing. During the parthenogenesis females produce unfertilised eggs from which hatch only females. During adverse environmental conditions (extreme temperatures, increase of population density, accumulation of excretion products, low food availability) also males are produced; these males fertilize particular eggs (resting eggs) that are then carried by females into a particular structure known as 'ephippium'. From these eggs hatch females that will reproduce again parthenogenetically.

Daphnia magna is utilized essentially because it is widely distributed in freshwaters and constitutes an important link in the food web being placed at an intermediate position between primary producers and fish consumers; furthermore, in some small ecosystems it is the final consumer (Müller, 1980). The breeding of *Daphnia magna* in the laboratory is easy and, thanks to its biological characteristics, it is possible to obtain easily a lot of specimens homogeneous for age and growth rate. Furthermore, thanks to the parthenogenesis it is possible to have identical individuals; this is a very important factor to minimize the individual variations in the response to toxicants. In addition, *Daphnia magna* has a quite short life cycle, thus it is possible to carry out fast chronic toxicity tests also for more generations.

In toxicity test with *Daphnia magna* two main parameters, mortality and immobilization, are recorded and the results are expressed respectively as LC_{50} and EC_{50}. Nevertheless, the parameter "immobilization" has been subject of criticism because a scarce mobility was observed in "sluggish" specimens which were motionless after stimulation, but subsequently can return to swim actively (Müller, 1980).

The tests with *Daphnia magna* must be carried out according to well-defined standards. According to IRSA-CNR (1994) the organisms must have homogeneous age (<24 hours), in general the tests should be conducted for 24-48 hours in static flux conditions, at 20°C and pH 7.5-8.5, with light-dark period 16 hrs - 8 hrs; the utilized standard water must have total hardness 140-160 mg $CaCO_3$/l, alcalinity 110-120 mg $CaCO_3$/l.

Recent methods were published by OECD: the method OECD 202 (2004) concerns the use of young daphnids, aged less than 24 hrs, and the exposition to different concentrations of toxicants for 48 hrs against control test. The immobilisation must be evaluated after 24 and 48 hrs and the results must be expressed as EC_{50}. Other daphnid species, such as *Daphnia pulex*, *Ceriodaphnia affinis* and *Ceriodaphnia dubia*, can be utilized in this test. The method OECD 211 (2008) concerns the evaluation of reproduction and utilizes specimens aged less than 24 hrs. The exposition is prolonged for 21 days and the living offspring produced is evaluated; survival, LOEC and NOEC are the common expression of results.

3.2 Marine crustaceans

Marine crustaceans useful for ecotoxicological testing are both benthic and planktonic and can be chosen mainly from adult and larval copepods, larval brine shrimps, larval barnacles and amphipods.

On the whole, *Artemia*, the brine shrimp typical of hypersaline waters, has been considered for long time the "standard" species (Carli et al., 1998) and has been currently used to evaluate the acute toxicity of several inorganic and organic contaminants (Baudo et al., 2011). *Artemia* specimens are in general easily available and the breeding does not show particular difficulties; these are certainly important factors to promote the utilization of this organism. As a matter of fact, it is normally easy to obtain many individuals starting from commercial cysts. In spite of this, to date the employment of *Artemia* is controversial particularly owing to its supposed inadequate sensitivity (Weideborg et al., 1997; Davoren et al., 2005). Otherwise, recent studies indicated that the evaluation of survival in *Artemia* in long-term toxicity tests is an useful and sensitive parameter (Brix et al., 2003, 2004; Manfra et al., 2009).

In marine ecotoxicology some copepods, such as the calanoid *Acartia tonsa* and the harpacticoids *Nitocra spinipes*, *Tisbe battagliai*, *Tigriopus fulvus* and other *Tigriopus* spp., and the amphipods *Corophium insidiosus*, *Corophium orientale* and *Corophium volutator* and other species indicated by ASTM (1999) seem to be eligible to play the role of test-species (Baudo et al., 2011) in order to support the brine shrimp *Artemia*, already extensively used, and to replace not easily available species, such as the mysid *Mysidopsis bahia*, an autochthonous species of Eastern coasts of North America, that was indicated in some regulations without considering the difficulties of its importing in the EU.

Amphipods are widely used in ecotoxicology, owing to their sensitivity to several contaminants such as metals (Zanders & Rojas, 1992; Liber et al., 2011; Mann et al., 2011; Strom et al., 2011), for the evaluation of sediments in marine and transition environments

(Chapman & Wang, 2001) and have been employed to draw up sediment-quality guidelines (Macdonald et al., 2011).

ASTM (1999) suggests for testing some amphipods species but unfortunately none of them occurs in the Mediterranean, making problematical their use for the laboratories of this region; on the whole, among the species considered in the guidelines the sole amphipod useful for the Mediterranean is *Corophium orientale* that is cited in the protocol ISO 16712 (2005). *Corphium orientale* has been indicated to be suitable in ecotoxicology mainly for its constant availability, for its high tolerance to the variations of salinity and for the reproducibility of given results that were verified comparing different populations sampled in Italian sites (Lera et al., 2008) but, in spite of this, the difficulties in sampling and breeding is a critical factor. As a matter of fact, to date the main problem for using of these amphipods concerns the impossibility to breed them; among the European amphipods suggested by OSPAR (1995) only *Corophium volutator* has been bred in the laboratory (Peters & Ahlf, 2005), but it does not occur in the Mediterranean.

Otherwise, harpacticoid copepods can be useful test-organisms for their wide distribution, their key-position within the food web, their satisfactory sensitivity to pollutants and because they are easier to rear than other crustaceans and also than pelagic copepods. Furthermore, the breeding of some harpacticoids allows to have many organisms that are always available owing to the constant and abundant production of offspring with very low costs and efforts. In some harpacticoids the production of offspring can be also stimulated. For these reasons harpacticoid copepods seem to be the chief candidate to hold the role of primary consumer in ecotoxicological testings.

To date the studies on the ecotoxicological response of *Acartia tonsa* and *Tigriopus fulvus*, two species eligible to the role of test-species in aquatic ecotoxicology, are in progress in Italy with the aim to contribute to the standardization of test methodologies; these studies are being carried out in the framework of an Italian inter-calibration programme including different laboratories using heavy metals as reference substances.

4. New approaches in marine ecotoxicology: promising copepod test-species

Copepods are emergent organisms in ecotoxicology; to date their employment is increasing even though for some species the availability is a critical factor; the main problem concerns the adequacy of the test-species in relation to their environment.

As stated above, the copepods used in marine ecotoxicology are essentially the calanoid *Acartia tonsa* and the harpacticoids *Nitocra spinipes*, *Tisbe battagliai* and *Tigriopus fulvus*; other copepods have been used sporadically.

In spite of this, the usefulness of *Tisbe battagliai* and *Nitocra spinipes* can be problematical in the EU (Baudo et al., 2011).

The calanoid *Acartia tonsa* Dana, 1846 is a small euryhaline and eurytherm copepod, it is widely spread and is typical of eutrophic coastal waters and harbours, as well as of estuaries and lagoons worldwide (Cervetto et al., 1995). It is known to be a cosmopolitan copepod and occurs mainly in waters with high trophism (Baudo et al., 2011). In the Mediterranean region *Acartia tonsa* was found first in late '80s of the last century (Farabegoli et al., 1989; Sei et al., 1996); it is supposed to be an allochtonous species for the Mediterranean where to date occurs mainly in the Adriatic Sea.

Acartia tonsa can be found in all seasons, with remarkable abundance from April to November (Baudo et al., 2011). During the last decade it has been widely employed in toxicity testing with several substances such as metals (Bielmyer et al., 2006), endocrine disruptors (Andersen et al., 2001; Kusk & Wollenberger, 2007), brominated compounds (Wollenberger et al., 2005), cosmetic and sunscreen components (Kusk et al., 2011), LAS (linear alkyl benzene sulfonate) (Christoffersen et al., 2003), insecticides (Barata et al., 2002; Medina et al., 2002) and other different chemicals (Sverdrup et al., 2002).

The specimens useful for the experiments can be collected with zooplankton nets provided with 50 – 200 µm mesh; to start breeding it is suggested to collect 200-300 adult *Acartia tonsa* (both males and females). Adult males and females *Acartia tonsa* can be recognized under a dissecting microscope following the indications of classical taxonomy (Rose, 1933) and removed using a wide-bore pipette (Buskey & Hartline, 2003; Invidia et al., 2004). Zooplankton samples must be maintained in appropriate recipients at 20°C ± 0.5°C with aeration; the sorting of specimens and the taxonomical recognition must be carried out as soon as possible and anyhow within few days from sampling. Subsequently, *Acartia tonsa* can be maintained in flow-through system with natural seawater at temperature 20 ± 2°C; it is necessary to provide constant aeration and a light/dark period 16/8 hrs, at 1800-2100 lux (Widdows, 1998). The organisms can be fed twice a week *ad libitum* with algae from batch cultures of different species such as *Isochrysis galbana* and *Tetraselmis suecica*.

Algal cultures used to feed copepods must be used at the exponential growth phase; suitable density to feed copepods can be 1.3 x 10^6 cells/ml for *Isochrysis g*albana and 0.35 x 10^6 cells/ml for *Tetraselmis suecica* supplying 7 ml/l *Isochrysis galbana* and 7 ml/l *Tetraselmis suecica*. The counting of algal cells can be performed by using a Thoma hemocytometer.

Toxicity tests must be performed using *Acartia tonsa* eggs obtained 15-16 hours before the test starting from the specimens maintained in the laboratory at the above described conditions.

The harpacticoid copepod *Tigriopus fulvus* assumed recently a pre-eminent role in ecotoxicology and demonstrated to be a promising target-species (Todaro et al., 2001; Faraponova et al., 2003; Pane et al., 2006a, 2006b). *Tigriopus fulvus* is the most representative organism in the splashpools of rocky Mediterranean littorals (Pane & Mariottini, 2010); it is adapted to live in pools located at different height above the tideline, characterized by wide salinity variations and also by mixing of marine and fresh waters, while it is absent in the pools reached by waves and in higher pools that receive almost exclusively the contribution of freshwater (Carli et al., 1995). Anyhow, as it is well known after observations in the laboratory, *Tigriopus fulvus* can survive normally both in natural and in artificial seawater (Carli et al., 1989a).

The specimens can be easily sampled in splashpools of rocky coasts; before testing they need to be acclimatized at least ten days at the laboratory conditions in filtered natural or artificial seawater at temperature 18.0±0.5 °C, 18 PSU (Practical Salinity Units) and neutral pH, with a 12/12 hrs light/dark period. The organisms must be fed once a week with algae from batch cultures (mainly *Tetraselmis suecica* or *Chlorella minutissima*) and bakers' yeast (*Saccharomyces cerevisiae*) counting the cells by a Thoma hemocytometer (Pane et al., 2008b).

The ecotoxicological tests can be carried out on adults (generally only females are used because in laboratory breeding they preponderate on males) and on the first larval stages (nauplii I-II) born in the laboratory culture; for tests on nauplii to have same-aged specimens is a very important factor in order to standardize the procedure. A simple method to obtain a suitable amount of same-aged nauplii provides for the isolation of carrying eggs females; subsequently the hatching of eggs must be stimulated by detaching egg sacs using fine needles after immobilization of females by soft filtration on membrane filters leaving a thin water film to avoid to damage them (Pane et al., 2006b). Detached egg sacs must be transferred into cell culture multiwell plates in seawater (18 PSU) and maintained at 18±0.5°C for 24 hours. Newborn nauplii (I-II stage) hatched by detached eggs, having the same age, can be utilized in toxicity tests.

The chronic tests on females, besides survival, consider also the production of egg sacs and of alive nauplii, so, they should be carried out preferably in multiwell plates with extractable polystyrene inserts provided with a membrane with pore mesh size 74 µm that allow to separate the females from offspring and to easily count the nauplii (Pane et al, 2008b).

Tigriopus fulvus has been extensively studied from the biological (Carli & Fiori, 1977; Carli et al., 1989a), biochemical (Carli et al., 1989b; Pane et al., 2003) and ecological point of view (Carli et al., 1993; Pane et al., 2000). Some studies have used this copepod to evaluate the toxicity of metals, surfactants, dispersants and other compounds of environmental concern (Giacco et al., 2006; Pane et al., 2007a, 2007b, 2008a, 2008b, 2009) in the framework of extensive experiments including the use of ecotoxicological test sets with several organisms (bacteria, algae, crustaceans, fish larvae).

Tigriopus fulvus has been recently included by the Italian Law among the species to be used to evaluate the suitability of natural or synthetic absorbent products and dispersants employed in seawaters for draining of oil hydrocarbon contamination (Gazzetta Ufficiale della Repubblica Italiana, 2011).

Other species of the genus *Tigriopus* have been considered for ecotoxicology: the first studies were made during the '70s and 80's of the last century when the effect of environmental contaminants, such as oil by-products, and pesticides was studied on *Tigriopus californicus* by Barnett and Kontogiannis (1975) and Antia et al., (1985) respectively, demonstrating the high adaptive capability of these copepods to the stress caused by xenobiotics.

Tigriopus brevicornis was utilized mainly to assess the toxicity of both essential and non-essential metals (Forget et al., 1998; Barka et al., 2001), considering also the detoxification processes (Barka, 2000, 2007) and the enzyme activity (Forget et al., 2003). Other studies concerned the effect of thermal shocks simulating the action of coastal nuclear power stations (Falchier et al., 1981) and the assessment of pesticide toxicity (Forget et al., 1998). Its role as water quality indicator has been also considered (Barka et al., 1997).

Tigriopus japonicus has been widely used in ecotoxicology and a lot of papers are available; recently the exposition to benzo(a)pyrene (Bang et al., 2009) and to alkylphenols (Hwang et al., 2010) was assessed on this copepod. Furthermore, the action of effluents in comparison with *Daphnia magna* (Kang et al., 2011), of metals (Ki et al., 2009; Kim et al., 2011; Kwok et al., 2008; Rhee et al., 2009) and the expression of glutathione S-transferase (Lee et al., 2007, 2008), the exposition to endocrine disruptors (Lee et al., 2006, Rhee et al., 2009) and to antifouling biocides (Kwok & Leung, 2005) have been also studied recently.

5. Conclusion

To date pollution rising from anthropogenic sources play an increasing environmental role. In addition, this phenomenon has an interest in all environments because pollution occurring in air, soil and freshwaters can be carried to seawaters and drained into coastal zones exerting toxicity on all organisms and persisting in time. Taking into account also the bioaccumulation processes, the monitoring of ecotoxicity is essential to determine the effects of pollutants at the global level. In this framework the availability of sensitive test-species is a very important aspect.

In this connection some crustaceans, for their wide distribution and for their key-position in the food web, have been proposed recently as test-species in ecotoxicology and the obtained results seem to be promising. In particular, the calanoid copepod *Acartia tonsa* and the harpacticoid copepod *Tigriopus fulvus* have shown to have several useful characteristics to play the role of test-species in ecotoxicology in the procedure of “risk assessment” concerning different chemicals.

In conclusion, in the framework of the REACH regulation further efforts are needing to adequate the research and the testing to the new regulations, taking also into account the need to prefer the use of invertebrates instead of vertebrates and, where possible, to replace the toxicity experiments with living organisms with alternative techniques, including analytical techniques useful for the screening of substances, predictive models and *in vitro* procedures.

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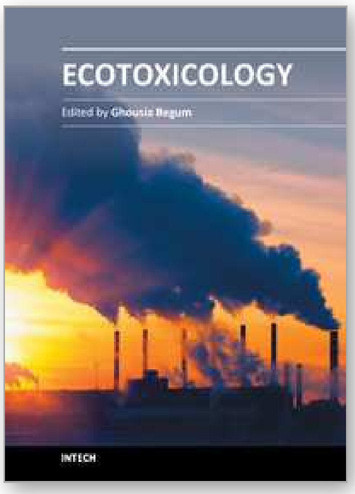

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This is a good book on upcoming areas of Ecotoxicology. The first chapter describes genotoxicity of heavy metals in plants. The second chapter offer views on chromatographic methodologies for the estimation of mycotoxin. Chapter three is on effects of xenobiotics on benthic assemblages in different habitats of Australia. Laboratory findings of genotoxins on small mammals are presented in chapter four. The fifth chapter describes bioindicators of soil quality and assessment of pesticides used in chemical seed treatments. European regulation REACH in marine ecotoxicology is described in chapter six. X-ray spectroscopic analysis for trace metal in invertebrates is presented in chapter seven. The last chapter is on alternative animal model for toxicity testing. In conclusion, this book is an excellent and well organized collection of up dated information on Ecotoxicology. The data presented in it might be a good starting point to develop research in the field of ECOTOXICOLOGY.

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