

Fungicides as Endocrine Disrupters in Non-Target Organisms

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1. Introduction

In the past few decades concern has been growing about the possible consequences of environmental exposure to a group of chemicals (natural, synthetic, industrial chemicals or by-products) suspected to alter the functions of the endocrine system and consequently causing adverse health effects in an intact organism, its offspring, or (sub) population (European Commission, 2007), the Endocrine Disruptor Compounds (EDCs). Today, this concern is focused both on human health and on the impacts on wildlife and the environment, being already a priority in research and legislation within the European Union (European Commission, 1999, 2001, 2004, 2007), the US Environmental Protection Agency (Kavlock et al., 1996; U.S. EPA, 1998; Harding et al., 2006) and the World Health Organization (Damstra et al., 2002).

All vertebrate and invertebrate taxa use chemical signalling molecules (hormones). Changes of the endocrine function can occur when EDCs interfere with the synthesis, secretion, transport, action or elimination of natural hormones, which are responsible for homeostasis mechanisms, reproduction, growth and/or behaviour. These interferences can be caused by the direct binding of EDCs to hormone receptors – acting as hormone mimics (agonists) or as "anti-hormones" (antagonists) – or indirectly through modulation of endogenous hormone levels by interfering with biochemical processes associated with the production, availability, or metabolism of hormones or also by the modulation of their receptors (Rodriguez et al., 2007).

2. Endocrine active compounds and invertebrates

Although invertebrates dominate over 95% of the known animal species and represent more than 30 different phyla within the animal kingdom (Ruppert et al., 2003), potential effects of suspected EDCs on the various invertebrate endocrine systems have not been studied with comparable intensity as in vertebrates, especially in fish (e.g. Baker et al., 2009), reptiles (e.g. De Falco et al., 2007), amphibians (e.g. Kaneko et al., 2008), birds (e.g. Halldin et al., 2001), and mammals (e.g. Tabuchi et al., 2006).

Even though the issue of Endocrine Disruption (ED) in invertebrates received some scientific interest in the past, only a limited number of confirmed cases were reported (deFur

et al., 1999). These are largely dominated by investigations on insect growth regulators (IGRs), which are designed to act as EDCs for insect pest control, and by studies on the antifouling biocide tributyltin (TBT) that has been shown to induce imposex and intersex in prosobranch snails (Matthiessen & Gibbs, 1998; Sousa, 2009b). Imposex has been associated with skewed sex ratios, reduced fecundity, population declines, and local extinctions of affected gastropod populations (Gibbs & Bryan, 1986). These are perhaps the most complete examples of ED studies in wildlife populations. Further examples for ED in invertebrates are scarce and limited to laboratory studies, where effects on endocrine regulated processes in marine and freshwater invertebrates (Porte et al., 2006), and in the soil compartment have been demonstrated for some compounds (Lemos et al., 2009, 2010a, 2010b, 2010c). Endocrine changes following exposure to certain compounds may therefore be missed or simply be immeasurable at present, even though there is increasing evidence indicating that invertebrates are susceptible to ED (Porte et al., 2006).

Consequently, there is no reason to suppose that far-reaching changes as demonstrated by TBT and its effects on prosobranch populations are in any sense unique within invertebrates (Matthiessen & Gibbs, 1998).

Additionally, since many chemicals have been considered as endocrine disrupters in vertebrates and chemical signalling systems and their basic mechanisms in the animal kingdom exhibit some degree of conservatism (McLachlan, 2001) we can presume that endocrine systems in invertebrates can be subject to modulation by identical or similar compounds as in vertebrates (Pinder and Pottinger, 1998). But, and as stated before, despite their abundance and variety, relatively little is known about their endocrine systems, making data obtained by studies on endocrine disruption rather difficult to interpret.

The “Endocrine Disruption in Invertebrates: Endocrinology, Testing, and Assessment” report (deFur et al., 1999) summarizes 56 studies where ED may have occurred in invertebrates, although non-endocrine mechanisms are also possible scenarios for the observed effects. Effects like reduced molting frequency, reduced fecundity, elevated ecdysteroid levels, delayed reproduction, reduced size of neonates, increased brood size, mortality, molting impairment, delayed maturation, impairment of reproduction, reduced egg production, delayed brood release, reduced elimination of testosterone metabolites, retardation of regenerative limb growth, suppression of ovarian growth, differential sex ratio and super-female induction, have been reviewed in this report. This includes several studies which comprise many compounds suspected of being hormonally active on aquatic crustaceans.

With the exception of TBT, effects in molluscs, that have been associated to a locally severe impact on community levels (e.g. Blaber, 1970), and IGRs in target terrestrial insects, there are only a few field examples of ED in invertebrates. Nevertheless, much more examples for ED affecting invertebrate populations and communities can be expected.

In fact, numerous studies provide strong evidence of effects on development, fecundity and reproductive output of invertebrates that can be attributed to substances acting as EDCs (Gibbs & Bryan, 1986; Matthiessen and Gibbs, 1998; Pinder and Pottinger, 1998; Oehlmann & Schulte-Oehlmann, 2003). So, carefully targeted monitoring programs are needed because effects in invertebrates are probably widespread but undetected (Fent, 2004).

3. Pesticides as Endocrine Disruptor Compounds

In June 2000 a list of 564 potential EDCs was published in two reports of BKH Consulting Engineers, Delft, and TNO Nutrition and Food Research, Zeist, both from The Netherlands (European Commission, 2000). This list of substances was compiled having in mind the compound persistence in the environment, its production volume, the scientific evidence of endocrine disruption and wildlife and human exposure. These criteria were used to categorise the candidate substances. From these, a group of 60 compounds considered to have endocrine disrupting activity (i.e. compounds for which endocrine activity has been shown in at least one *in vivo* study) and for which a high level of concern existed with regard to exposure, deserved a special attention. These 60 compounds were included in a high priority list of EDCs proposed by the EU Commission (European Commission DG ENV, 2002). This list includes industrial chemicals such as plasticizers (e.g. benzyl-n-butylphthalate, di-n-butylphthalate, bisphenol A) or flame retardants (e.g. PBBS) but also agrochemicals or crop protection products (e.g. lindane, vinclozolin, linuron, diuron, the common metabolite of linuron and diuron, 3,4-dichloroaniline, as well as triphenyltin compounds), and biocides with antifouling properties (tributyltin compounds).

4. Fungicides as endocrine-disrupters

The increased need for pest control has made pesticide use a major issue in environmental risk assessment. Within these compounds, despite some ecotoxicology studies reporting their low toxicity to non-target species (Jansch et al., 2005; Haeba et al., 2008), many fungicides have already been reported as affecting organisms and their molecular targets and effects are now well documented.

It is not surprising that several examples of endocrine disruption have been reported for terrestrial arthropods, as several pesticides have been specially tailored to affect insects endocrine systems (IGRs), eventually co-affecting several non-target invertebrates (deFur et al., 1999). In aquatic environments the examples are scarce and reduced to the effects of TBT in molluscs that have been associated with a locally severe impact at the marine community levels (Matthiessen & Gibbs, 1998).

For this review two compounds from the EU highest priority list were selected based on their environmental occurrence as well as the existing studies confirming ED effects: the fungicides tributyltin (androgen) and vinclozolin (anti-androgen). Focusing on these compounds and their effects on non-target invertebrates, an overview of terrestrial and aquatic compartments is here addressed.

5. Case studies

5.1 Tributyltin

Tributyltin (TBT) compounds are a subgroup of the trialkyl organotin family of compounds. Of all known organotins, some of the most toxic are tributyltin compounds like tributyltin oxide (TBTO; Fig.1A) and tributyltin chloride (TBTCl; Fig.1B) (Carfi 2008). TBT compounds are organic derivatives of tin (Sn^{4+}) characterized by the presence of covalent bonds between three carbon atoms and a tin atom (Antizar-Ladislao, 2008). While inorganic forms of tin are regarded as non-toxic, these more lipid-soluble organotins can be highly toxic (Gadd, 2000).

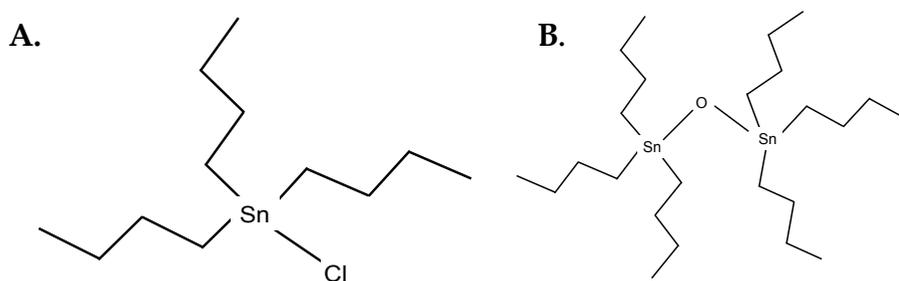


Fig. 1. Molecular structures of tributyltin chloride (A) and tributyltin oxide (B).

They are the main active ingredients in pesticides used to control a broad spectrum of organisms as they act as biocides for fungi, bacteria and insects (Mimura et al., 2008; Fent, 2006). Nevertheless, research undertaken since the early 1970s has shown that TBT is highly toxic to a larger number of non target aquatic organisms (Antizar-Ladislao, 2008). TBTs' properties were recognized in the 1950s and since then this compound has been extensively used for various industrial purposes such as slime control in paper, as a wood preservative, as a polyvinyl chloride (PVC) stabilizer, and as fungicide in agriculture (Mimura et al., 2008). In the 1970s, TBT paints widely replaced copper-based paints due to their superior performance in terms of efficacy and duration (Sonak, 2009). Since then, TBT has been used mostly as an antifouling agent in marine paint formulations to prevent the attachment of barnacles and slime on boat hulls and aquaculture nets (Kannan et al., 1998).

Due to its widespread use as an antifouling agent in boat paints, TBT is a common contaminant of marine and freshwater ecosystems. Its damaging consequences to marine ecosystems were recognized in early 1980s as the cause for the decline of some marine molluscs (Smith, 1981; Waldock & Thain, 1983; Bryan et al., 1986). As a result of field evidences of negative ecological impact of organotin, the European Union published a Directive (89/677/CEE) banning TBT application on ships smaller than 25 m. On the assumption that TBT concentrations in the open sea were too low to cause effects, there were not many restrictions on the use of organotin in larger ships. However, a similar impact in the open sea has been shown for TBT with incidence being correlated with shipping density (Santos et al., 2002). Thus, the International Maritime Organization banned the application of TBT-based paints in 2003 and called for a global agreement for total prohibition of the presence of organotin on ship hulls in 2008 (International Maritime Organization, 2001).

TBT from hulls and nets can be adsorbed onto suspended particles in the water, sediment and biota (Gadd, 2000). Subsequently it is readily incorporated into the tissues of filter-feeding zooplankton, invertebrates and eventually higher organisms such as fish and mammals where it accumulates (Antizar-Ladislao, 2008). Despite the present restrictions, TBT and its degradation products will not disappear immediately from the marine environment, and it can be expected that TBT will remain in waters and sediments for long periods of time because of the moderate to high persistency in anoxic sediments and its' widespread presence, as was confirmed by the work of Sousa and co-workers along the Portuguese coast (Sousa et al., 2009). Together with its lipophilicity, it tends to accumulate in oysters, mussels, crustaceans, molluscs, fish, and algae favouring the bioconcentration up the marine predators' food chain (Santos et al., 2009; Cruz et al., 2010).

Because of all the above mentioned, recently organotins have been considered as the most toxic substance ever introduced into the marine environment so far (Fent, 2006; Guo et al., 2010; Antizar-Ladislao, 2008; Sonak, 2009), and due to their use in a variety of industrial processes, their environmental fate and ecotoxicity as well as human exposure are topics of current concern.

In a study conducted by Guo et al (2010) on western clawed frog embryos (*Xenopus tropicalis*) the authors suggested that TBT might be the cause of several malformations. These include the loss of eye pigmentation, enlarged trunks and bent tails, in the presence of 50 ng/L of TBTCI after 24 hours of exposure. This is particularly relevant since the concentrations of TBT in open water, bays, estuaries, lakes and freshwater harbors commonly exceed this concentration with the highest TBT values found near marinas and seaports (Fent, 2006). At higher concentrations TBT may be lethal to several marine and freshwater species. Short and Thrower (1987) reported that the 96 hour LC50 for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) is 1.5 µg/L.

TBT is known as an endocrine disruptor promoting adverse effects in organisms on diverse levels of biological organization (Guo et al., 2010). Matthiessen & Gibbs (1998) reported an interference with hormone metabolism, increasing the androgen levels of snails exposed to TBT. One of the best-documented and iconic adverse impacts of TBT in non-target organisms is imposex in molluscs. This pathology occurs when male sex characteristics and organs, such as penis, *vas deferens*, and seminiferous tubules are superimposed on normal female resulting in female sterilization, and even with spermatogenesis occurrence. Such pathology consequently has obvious impacts population dynamics (Gibbs et al. 1991). The first evidences linking TBT to imposex were reported in 1970 for the dog-whelk, *Nucella lapillus*, in the UK (Blaber, 1970). Since then, several studies have related TBT to the worldwide decline of marine mollusks (e.g. *Nassarius*, *Ilyanassa*, *Ocenebra* and *Urosalpinx*) in coastal areas due to imposex (Gibbs et al., 1991). Administering testosterone to the snail *Euchadra peliomphala* resulted in a stimulation of the development of male sex characters in female and castrated male gastropods (Takeda, 1980; Spooner et al., 1991). These authors also report increased testosterone titres in *N. lapillus* exposed to this fungicide. These findings have led to the hypothesis that the increased levels of testosterone in TBT-exposed organisms are responsible for the imposex development. The precise mechanism by which increased levels of testosterone are produced has not been fully described, but the weight of evidence suggests that TBT acts as a competitive inhibitor of cytochrome P450-mediated aromatase (Bettin et al., 1996). In laboratory tests, imposex was also reported from concentrations below 1 ng of TBT/L in the mud snail, *Ilyanassa obsoleta* (Gooding et al., 2003), and the dog-whelk, *N. lapillus* (Gibbs et al., 1988). There are still uncertainties regarding the sensitivity of the endocrine function in different genders and developmental stages in invertebrates exposed to EDCs (Rodriguez et al., 2007). Nevertheless, some authors agree that the hormonal impacts of TBT and EDCs in general may differ according to the specific life stage at which exposure occurs (e.g., embryolarval stages, gonadal development, etc.) (deFur et al., 1999; Lemos et al., 2009). Generally, the larvae/neonates of any tested species are more hormonally sensitive to tributyltin exposure than are the adults. For example, the adult female dog-whelk, *N. lapillus*, revealed imposex signs at concentrations as low as 5 ng of TBT/L while young and sexually immature females were more sensitive than adults with concentrations of 1 ng of TBT/L inducing the growth of penis and *vas deferens* tissue (Gibbs et al., 1987).

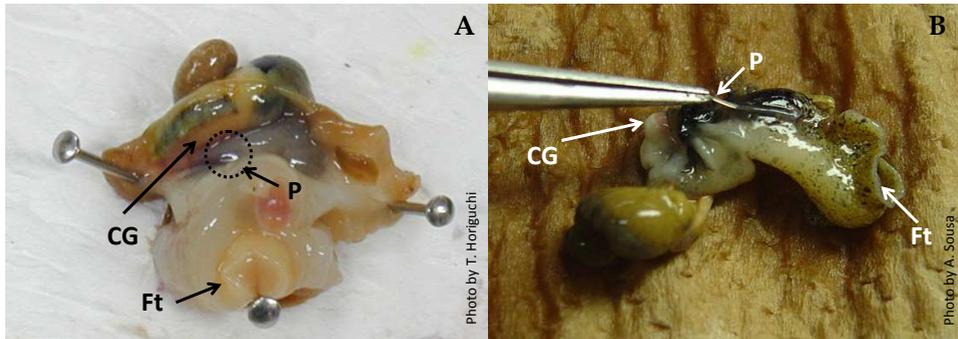


Fig. 2. Female gastropods exhibiting imposex: *Nucella lapillus* (A), and *Nassarius reticulatus* (B). CG - capsule gland; Ft - foot; P - penis. Photographs are courtesy of Prof. T. Horiguchi and Dr. A. Sousa.

Deviations from normal limb regeneration in the prawn, *Caridina rajadhari* (Reddy et al. 1991), and in the fiddler crab *Uca pugilator* (Weis et al., 1987), have been observed in laboratory experiments involving TBT exposure. Nevertheless, despite the findings and the known hormonal regulation of the molting process, it has been argued whether these effects are due to endocrine disruption rather than regular systemic toxicity. Unless specific parameters such as hormone levels are assessed no accurate conclusion can be drawn (Barata et al., 2004; Lemos et al 2009).

Levels in harbour and port waters prior to restrictions on TBT use in antifouling paints have shown levels higher than 500 ng/L in North American and European marinas. For example, one year before the UK ban (1986), TBT concentrations in Wroxham Broad and at the nearby River Bure boatyard were 898 ng/L and 1540 ng/L, respectively (Waite et al., 1989). They were significantly higher than in open surface waters, bays and estuaries where commonly values of up to 50 ng/L were observed (Fent, 2006). Albeit this regulation for the use of TBT and consequent general decrease in environmental TBT levels (Antizar-Ladislao, 2008), recent surveys still account for levels higher than those reported to elicit effects at a global scale: for instance, 32 ng TBT/L in South Korea (Sidharthan et al., 2002), 2-160 ng/L in Japanese coastal waters (Takeuchi et al., 2004), in the UK 10-78 ng/L were detected in marinas and harbors in 1998 (Thomas, 2001), and 200 ng/L in ferry ports in Corsica, Italy (Michel et al., 2001).

The impact of TBT in freshwater systems has been studied at a much lesser extent compared to estuarine and coastal environments. As still, endocrine effects have also been shown for freshwater molluscs. For example, after a 3 month exposure to a concentration of 50 ng of TBT/L, the giant ramshorn snail, *Marisa cornuarietis*, showed recognizable morphological characteristics of imposex development (Shulte-Oehlmann et al., 1995). Despite the scarce information (possibly reflecting a reduced concern about the impacts in this ecosystem), Schulte-Oehlmann (1997) reported concentrations in European lakes up to 930 ng of TBT /L in water and reaching 340 µg of TBT/g wet weight in sediments of River Elbe in Central Europe.

For the soil compartment, to our knowledge, there are no reported data on the effects of TBT to edaphic organisms.

5.2 Vinclozolin

Vinclozolin [Vz, 3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione; Fig.3] is a non-systemic dicarboxymide fungicide, manufactured by BASF and commercially sold under the names Ronilan[®], Curalan[®], and Ornilan[®]. It is efficient in controlling plants and fruit diseases caused by *Botrytis* spp., *Monilia* spp., and *Sclerotinia* spp. (Bursztyka et al., 2008) that affect crops such as lettuce, raspberries, beans and onions (Price et al., 2007). This fungicide is widely used in the United States of America and throughout Europe. In Britain, as well as in Germany, up to 50 tonnes of Vz are used each year and it was estimated that in 2002, in the USA, 2,330,738 US dollars were spent on this compound for crop protection (Gianessi & Reigner, 2005).

When sprayed as Ronilan[®], at the maximum recommended application rate, the concentration of Vz in the soil is 1 mg active ingredient (a.i.)/kg (assuming that 70 % of the fungicide will reach the surface and is homogeneously distributed over the top 5 cm soil layer and the soil bulk density is 1.4 kg/dm³) (Lemos et al., 2009). Vinclozolin has a low to moderate persistence in soil, with reported half-lives from 28-43 days in the laboratory up to 34-94 days in the field and 6-12% of the original compound is present after 1 year (U.S. EPA, 1991; IUPAC, 2006). Despite this low-persistence of Vz, a reported increase of mortality with exposure time (Lemos et al., 2009) may be due to the increased concentration of its' three major metabolites (out from 15): 2-[[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenic acid (M1), 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), and 3,5-dichloroaniline (DCA), which are reported to be more active than the parent compound (Kelce et al., 1997; Anway et al., 2005; Kavlock and Cummings, 2005) and have half-lives ranging from 179 to >1000 days (U.S. EPA, 2000). It has been reported that these metabolites may be produced both spontaneously in the presence of aqueous buffers and by biotransformation of Vz (Bursztyka et al., 2008). M1 and M2 are able to bind to the androgen receptor (Vinggaard et al., 1999) and competitively inhibit the binding of androgens to the human androgen receptor (Kelce et al., 1997; Kavlock and Cummings, 2005). Concerning the parent compound, it is known that it inhibits vertebrate testosterone-induced growth of androgen-dependent tissues (Kang et al., 2004).

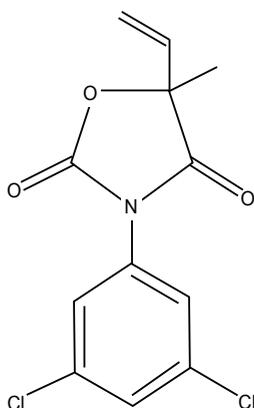


Fig. 3. Molecular structure of vinclozolin.

On plant leaves, Vz is detectable as the parent compound but does not wash off, since it is more soluble in oil than in water. This implies that Vz residues are commonly found on food (Szeto et al., 1989). Metabolites have also been found in human food (Gonzalez-Rodriguez et al., 2008).

Vinclozolin endocrine disruptor effects include induction of Leydig cell tumors, reduction of ejaculated sperm counts and prostate weight, and delayed puberty in exposed rats. One major concern is that Vz causes transgenerational effects. F1 to F4 generations of male rats exposed to Vz at the time of gonadal sex determination developed prostate disease, kidney disease, immune system abnormalities, spermatogenesis abnormalities, breast tumour development, and blood abnormalities as hypercholesterolemia, which have been associated with an alteration in the epigenetic programming of the male germ line (Anway et al., 2006; Anway & Skinner, 2008). Similar effects have been shown for pregnant rat exposed to Vz, where a transgenerational increase in pregnancy abnormalities and female adult onset disease states are promoted (Nilsson et al., 2008).

The existing information supports the hypothesis that Vz steroid-mediated actions in vertebrates have similar sub-lethal effects in invertebrates. In *Daphnia magna* it induces a decrease in the number of newborn males (Haeba et al., 2008). In molluscs Vz was shown to cause female virilisation (imposex development) and reduction of accessory sex organ expression in the fresh water snail *Marisa cornuarietis* and two marine prosobranchs *N. lapillus* and *Nassarius reticulatus* (Tillmann et al., 2001). Snails were exposed to nominal concentrations of Vz ranging from 0.03 to 1 µg/L for up to 5 months. In exposed juvenile *Marisa*, males had a slight decrease in the male accessory sex organ, particularly the penis and penis sheath. This response was only detected during the first 2-3 months of exposure for the lowest concentrations (0.03 and 0.1 µg of Vz/L), and was reversible once they attained puberty. Adult male *N. lapillus* exposed to the fungicide developed shorter penis, smaller prostate gland, and there were less males with ripe sperm stored in the seminal vesicle. Compared to the reported effects of estrogens and androgens on these two same species, these anti-androgenic responses seem to be less drastic, and might not have any biological effect at the population level (Tillmann et al., 2001). The immobilising effect (EC50, 48h) of Vz on the American oyster (*Crassostrea virginica*) and *D. magna* was reported to be 3.2 mg/L and 3.65 mg/L, respectively. For the opossum shrimp (*American bahia*) the LC50 (96h) was 1.5 -2.1 mg Vz/L (U.S. EPA, 2000).

Studies of Vz effects in the soil compartment are scarce. Vinclozolin has been reported as non-toxic to earthworms (Tomlin, 2003), but the most complete and extensive research was performed in a terrestrial isopod (Lemos et al. 2009, 2010a, 2010b, 2010c). In *Porcellio scaber*, Vz exhibited ecdysteroidal activity in ecdysone endogenous levels in a concentration-dependent way (Lemos et al., 2009). The results from this study demonstrated that the fungicide caused endocrine disruption in the isopod with an ecdysteroid up-regulation resulting in molting disturbances being further related to developmental and reproductive toxicity which enabled to suggest a causal link to ED in this class of organisms.

Since a sharp rise of ecdysteroid followed by a decrease of the hormone level triggers the ecdysis process (Bodar et al., 1990), when basal concentration of 20-hydroxyecdysone (20E) is maintained at high levels (hyperecdysonism) the shedding of the old cuticle is impaired and mortality due to incomplete ecdysis occurs (Fig. 4). Therefore, molting behaviour was associated with hyperecdysonism, delaying molt and in many cases impairing the molting process, and death surmounting at higher toxicant concentrations (1 g of a.i. Vz/kg of soil) (Lemos et al., 2009).

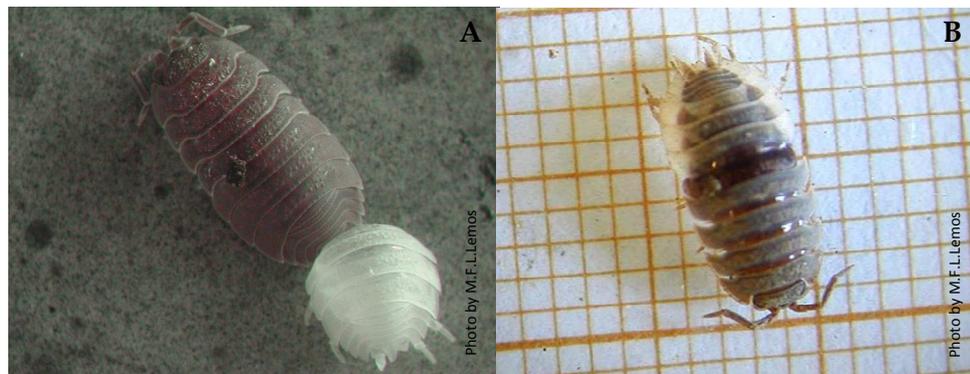


Fig. 4. Isopod *Porcellio scaber* and shedded cuticle after posterior half molt (A), and dead animal within the unshedded old cuticle after failed posterior half molt, after exposure to vinclozolin contaminated soil (B).

The same authors (Lemos et al. 2010a) also reported that young isopods respond with reduced growth at lower concentrations (LOEC of 100 mg a.i. Vz/kg soil) compared to adults (LOECs 300 mg a.i. Vz/kg soil). The increased sensitivity of the juvenile life stage may be either due to easier absorption of the toxicant through their relatively larger body surface/volume ratio and thin cuticle, or due to their lower capacity to metabolize the contaminants (Fischer et al., 1997; Lemos et al., 20010a) but another possibility pointed was the differential life-stage endocrinology.

Vinclozolin also elicited overall reproductive toxicity to *P. scaber* (Lemos et al., 2010b) decreasing the reproductive allocation for exposed females, the number of pregnancies, and the number of juveniles, while increasing the percentage of abortions. It induced a decrease of the brood period, with the isopods releasing juveniles almost 43 hours earlier at 100 mg a.i./kg dry soil and five days earlier at 300 mg a.i./kg dry soil. These two factors together considerably reduced the total juvenile output.

One of the reasons attributed to this impairment was the increased 20E titres (Lemos et al., 2009) that have been previously correlated with increased vitellogenin - a key protein of extreme importance in crustacean reproduction - synthesis and uptake in developing oocytes (Gohar & Souty, 1984).

The molecular effect of Vz was assessed by differential protein expression of Vz exposed *P. scaber* in the gut, hepatopancreas, and gonads (Lemos et al., 2010c). In this study it was possible to detected up-regulated proteins at concentrations as low as 10 mg a.i./kg soil in the testes while for hepatopancreas this was only possible at concentrations equal to 1000 mg a.i./kg soil. Proteins from the heat shock protein family, Hsp70 (known as ubiquitous stress response proteins, anti-apoptotic, and protects cells from cytotoxicity and inhibiting cell death induced by several agents) were over-expressed at the lower concentration of Vz in the testes of organisms exposed to Vz (around 160% increase after exposure to 10 mg a.i./kg and around 130% at 30 mg a.i./kg).

Vinclozolin up-regulated arginine kinase (around 150% at 1000 mg a.i./kg for Vz) in the isopods' hepatopancreas. This enzyme is involved in the cellular energy metabolism,

suggesting an increase of resources allocated to the activation of metabolic processes related to detoxification and the metabolisation of energy reserves to provide for these processes (Lemos et al., 2010c).

The fact that the gonads showed increased protein expression at concentrations higher than 10 mg a.i./kg soil (Lemos et al., 2010c), suggests that testes are more susceptible to these compounds than other organs. Thus, male isopod reproductive traits may therefore be especially susceptible and sensitive to this fungicide. Previous studies have also stressed the gonads and reproductive traits as preferential targets by EDCs in vertebrates (Navas and Segner, 2006).

6. Conclusions

Although TBT levels have been decreasing in the last decades, mainly due to restrictions in its use, it is still present at ng/L levels in the environment (Fent, 2006). Additionally, marine prosobranch gastropods and other invertebrates are extremely sensitive to TBT contamination, and imposex can be elicited in some species at concentrations of < 1 ng TBT/L (Gibbs et al. 1988). Moreover, TBT is very persistent in sediments being considered as the most toxic substance ever introduced into the marine environment and a major threat to the environment for many years (e.g. Fent, 2006) since its sediment concentrations (up to several mg/kg sediment) are still highly toxic to benthic fauna while organotins resuspension is possible through storms or dredging leading to an increase of organotin levels in the water column (Unger et al., 1988). Additionally one should not forget that organotins residues such as TBT can also be transferred to humans via dietary uptake. Sousa and co-workers predict that in traditional seafood-consuming countries the estimated Sn daily intake is high enough to cause damage to humans (Sousa et al., 2009b), making TBT contamination more than “just” an environmental issue of concern.

Vinclozolin is classified as very toxic (EC50 < 1 mg/L), and it has been shown to have adverse effects in the laboratory on aquatic snail at concentrations below 1 µg/L. Nevertheless, Vz is non-persistent in the environment, and degrades rapidly, particularly under alkaline conditions (Tomlin, 2003; Ueoka *et al.*, 1997). Moreover, a survey of German ground and surface waters found only 1% of water samples to have detectable levels (>0.1 µg/L) of Vz (Funari *et al.*, 1995). Mediterranean estuarine waters have also been investigated for fungicide contamination, and from the sites examined only the River Po in Italy had detectable levels of vinclozolin (Readman *et al.*, 1997).

In the soil compartment, despite these reported severe effects in isopods, the concentrations that elicit effects are far from ecological relevant concentrations.

So far there is no clear evidence suggesting effects of Vz on the terrestrial or aquatic ecosystems. Nevertheless, in Europe, the use of this substance is no longer authorized according to EC Directive 91/414.

Despite the examples showing that invertebrates are susceptible to ED, endocrine changes following exposure to certain compounds may be missed due to scarce knowledge of invertebrate endocrinology or due to very low concentrations of certain compounds (below detection limits) eliciting ED effects. In fact there is no reason to suppose that the array of endocrine changes such as the ones demonstrated for TBT and Vz are in any sense unique

and most certainly similar effects are plausible and to be expected in most invertebrate species.

Through small biochemical and molecular changes, these contaminants may however interfere with different systems (e.g., reproductive, endocrine, immunological, and nervous) in different life stages of non-target species, causing medium and long term effects at the population level. Due to the nature of the ED mode of action, the consequences of the exposure to such class of compounds in the communities' structure and function are thus not always immediate and can be extremely hard to predict.

As said before many fungicides can behave like EDC, and because ED effects at the population and community levels might be only detected after several generations exposed to sub-lethal levels of pollutants, there is a need to develop and validate quantifiable tools for the identification of ED effects of fungicides and pesticides in general. It is our conviction that this has to be achieved with a mechanistic approach where alterations of hormonal vital processes in particular species are assessed. For that it is vital to conduct long term chronic assays where biochemical, organismal and reproductive parameters are measured and compared with changes in hormone levels of invertebrate species. It is not an easy task but due to the expected increase in fungicide production and utilization in the next decades, it is critical to adopt this kind of integrative approaches to better understand the mechanisms that link endocrine level responses to population- and community-level processes and to improve environmental risk assessment of these compounds

7. Acknowledgements

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8. References

- Antizar-Ladislao, B. (2008). Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment: a review. *Environment International*, 34, pp. 292-308.
- Anway, M.D., Cupp, A.S., Uzumcu, M., & Skinner, M.K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*, 308, pp. 1466-1469.
- Anway, M.D., Skinner, M.K. (2008). Transgenerational effects of the endocrine disruptor vinclozolin on the prostate transcriptome and adult onset disease. *Prostate*, 68, pp. 517-529.
- Baker, M.E., Ruggeri, B., Sprague, L.J., Eckhardt-Ludka, C., Lapira, J., Wick, I., Soverchia, L., Ubaldi, M., Polzonetti-Magni, A.M., Vidal-Dorsch, D., Bay, S., Gully, J.R., Reyes, J.A., Kelley, K.M., Schlenk, D., Breen, E.C., Sasik, R., & Hardiman, G. (2009). Analysis of endocrine disruption in Southern California Coastal fish using an aquatic multispecies microarray. *Environmental Health Perspectives*, 117, pp. 223-230.

- Barata, C., Porte, C., & Baird, D.J. (2004). Experimental designs to assess endocrine disrupting effects in invertebrates - A review. *Ecotoxicology*, 13, pp. 511-517.
- Bettin, C., Oehlmann, J., & Stroben, E. (1996). Induced imposex in marine neogastropods is mediated by an increasing androgen level. *Helgoländer Meeresunters* 50, pp. 299-317
- Blaber, S.J.M. (1970). The occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus* (L.). *Journal of Molluscan Studies*, 39, pp. 231-233.
- Bodar, C.W.M., Voogt, P.A., & Zandee, D.I. (1990). Ecdysteroids in *Daphnia magna* - Their role in molting and reproduction and their levels upon exposure to cadmium. *Aquatic Toxicology*, 17, pp. 339-350.
- Bryan, G.W., Gibbs, P.E., Hummerstone, L.G., & Burt, G.R. (1986). The decline of the gastropod *Nucella lapillus* around south-west England: evidence for the effects of tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, 66, pp. 611-640.
- Bursztyka, J., Debrauwer, L., Perdu, E., Jouanin, I., Jaeg, J.P., & Cravedi, J.P. (2008). Biotransformation of vinclozolin in rat precision-cut liver slices: Comparison with in vivo metabolic pattern. *Journal of Agricultural and Food Chemistry*, 56, pp. 4832-4839.
- Carfi, M., Croera, C., Ferrario, D., Campi, V., Bowe, G., Pieters, R., & Gribaldo, L. (2008). TBTC induces adipocyte differentiation in human bone marrow long term culture. *Toxicology*, 249, pp. 11-18.
- Cruz, A., Caetano, T., Suzuki, S., & Mendo, S. (2007). *Aeromonas veronii*, a tributyltin (TBT)-degrading bacterium isolated from an estuarine environment, Ria de Aveiro in Portugal. *Marine Environmental Research*, 64, pp. 639-50.
- Damstra, T., Barlow, S., Bergman, A., Kavlock, R., & Van Der Kraak, G. (2002). Global Assessment of the State-of-the Science of Endocrine Disruptors. WHO/PCS/EDC/02.2. World Health Organisation, International Programme on Chemical Safety, Geneva, Switzerland.
- De Falco, M., Sciarillo, R., Capaldo, A., Russo, T., Gay, F., Valiante, S., Varano, L., & Laforgia, V. (2007). The effects of the fungicide methyl thiophanate on adrenal gland morphophysiology of the lizard, *Podarcis sicula*. *Archives of Environmental Contamination and Toxicology*, 53, pp. 241-248.
- deFur, P.L., Crane, M., Ingersoll, C., & Tattersfield, L. (1999). Endocrine disruption in invertebrates: endocrinology, testing, and assessment. *Proceeding of the Workshops on Endocrine Disruption in Invertebrates*, 12-15 December 1998, Noordwijkerhout, The Netherlands. SETAC Press, Pensacola, U.S.
- European Commission (1999). Communication from the Commission to the Council and the European Parliament - Community Strategy for Endocrine Disruptors. COM(1999)706. Commission of the European Communities, Brussels, Belgium.
- European Commission (2000). Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption - preparation of a candidate list of substances as a basis for priority setting. BKH-RPS Group, The Netherlands. P. 29.
- European Commission (2001). Communication to the Council and the European Parliament on the implementation of the Community Strategy for Endocrine Disruptors - a

- range of substances suspected of interfering with the hormone systems of humans and wildlife. COM(2001)262. Commission of the European Communities, Brussels, Belgium.
- European Commission (2004). Commission Staff Working Document on implementation of the Community Strategy for Endocrine Disrupters - a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706). SEC(2004) 1372. Commission of the European Communities, Brussels, Belgium.
- European Commission (2007). Commission Staff Working Document on the implementation of the "Community Strategy for Endocrine Disrupters" - a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706), (COM (2001) 262) and (SEC (2004) 1372). SEC(2007) 1635. Commission of the European Communities, Brussels, Belgium.
- European Commission DG ENV (2002). Study on gathering information on 435 substances with insufficient data. BKH-RPS Group, The Netherlands. p. 52.
- Fent, K. (2004). Ecotoxicological effects at contaminated sites. *Toxicology*, 205, pp. 223-240.
- Fent, K. (2006). Worldwide occurrence of organotins from antifouling paints and effects in the aquatic environment. *The Handbook of Environmental Chemistry*, 5, pp. 71-100.
- Fischer, E., Farkas, S., Hornung, E., & Past, T. (1997). Sublethal effects of an organophosphorous insecticide, dimethoate, on the isopod *Porcellio scaber* Latr. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology*, 116, pp. 161-166.
- Funari, E., Donati, Sandroni, D., & Vighi, M. (1995). Pesticide levels in groundwater: value limitations of monitoring. In: *Pesticide risk in groundwater*, Vighi, M. & Funari, E. (eds.), pp- 3-33. CRC press. Boca Raton, FL, U.S.
- Gadd, G. (2000). Microbial interactions with tributyltin compounds: detoxification, accumulation, and environmental fate. *The Science of The Total Environment*, 258, pp. 119-127.
- Gianessi, L.P., Reigner, N. (2005). The Value of Fungicides In U.S. Crop Production. CropLife Foundation - Crop Protection Research Institute. Washington, DC, U.S.
- Gibbs, P.E., Bryan, G.W. (1986). Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, 66, pp. 767-777.
- Gibbs, P.E., Bryan, G.W., Pascoe, P.L., & Burt, G.R. (1987). The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. *Journal of the Marine Biological Association of the United Kingdom*, 67, pp. 507 - 523.
- Gibbs, P.E., Pascoe, P.L., & Burt, G.R. (1988). Sex change in the female dogwhelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, 68, pp. 715-731.
- Gibbs, P.E., Bryan, G.W., & Pascoe, P.L. (1991). Tributyltin-induced imposex in dogwhelk, *Nucella lapillus*: Geographical uniformity of the response and effects. *Marine Environmental Research*, 32, pp. 79-87.
- Gohar, M., Souty, C. (1984). The temporal action of ecdysteroids on ovarian protein-synthesis invitro in the terrestrial Crustacean Isopoda, *Porcellio ditatatus* (Brandt). *Reproduction Nutrition Development*, 24, pp. 137-145.

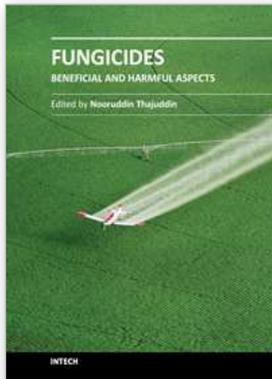
- Gonzalez-Rodriguez, R.M., Rial-Otero, R., Cancho-Grande, B., & Simal-Gandara, J. (2008). Determination of 23 pesticide residues in leafy vegetables using gas chromatography-ion trap mass spectrometry and analyte protectants. *10th International Symposium on Advances in Extraction Techniques*, Bruges, Belgium, pp. 100-109.
- Gooding, M.P., Wilson, V.S., Folmar, L.C., Marcovich, D.T., & LeBlanc, G.A. (2003). The biocide tributyltin reduces the accumulation of testosterone as fatty acid esters in the mud snail (*Ilyanassa obsoleta*). *Environmental Health Perspectives*, 111, pp. 426-430.
- Guo, S.Z., Qian, L.J., Shi, H.H., Barry, T., Cao, Q.Z., & Liu, J.K. (2010). Effects of tributyltin (TBT) on *Xenopus tropicalis* embryos at environmentally relevant concentrations. *Chemosphere*, 79, pp. 529-533.
- Haeba, M.H., Hilscherova, K., Mazurova, E., & Blaha, L. (2008). Selected endocrine disrupting compounds (vinclozolin, flutamide, ketoconazole and dicofol): Effects on survival, occurrence of males, growth, molting and reproduction of *Daphnia magna*. *Environmental Science and Pollution Research*, 15, pp. 222-227.
- Halldin, K., Berg, C., Bergman, A., Brandt, I., & Brunstrom, B. (2001). Distribution of bisphenol A and tetrabromobisphenol A in quail eggs, embryos and laying birds and studies on reproduction variables in adults following in ovo exposure. *Archives of Toxicology*, 75, pp. 597-603.
- Harding, A.K., Daston, G.P., Boyd, G.R., Lucier, G.W., Safe, S.H., Stewart, J., Tillitt, D.E., & Van der Kraak, G. (2006). Endocrine disrupting chemicals research program of the US Environmental Protection Agency: Summary of a peer-review report. *Environmental Health Perspectives*, 114, pp. 1276-1282.
- International Maritime Organization (2001). Final Act. International Conference on the Control of Harmful Anti-Fouling Systems for Ships, 1-5 October 2001. Report No. AFS/CONF/25. London: International Maritime Organization.
- IUPAC, 2006. *Global availability of information on agrochemicals: vinclozolin* (Ref: BAS 352F), accessed in 20 of July 2011, available from: <http://sitem.herts.ac.uk/aeru/iupac/680.htm>
- Jansch, S., Garcia, M., & Rombke, J. (2005). Acute and chronic isopod testing using tropical *Porcellionides pruinosus* and three model pesticides. *European Journal of Soil Biology*, 41, pp. 143-152.
- Kaneko, M., Okada, R., Yamamoto, K., Nakamura, M., Moscom, G., Polzonetti-Magni, A.M., & Kikuyama, S. (2008). Bisphenol A acts differently from and independently of thyroid hormone in suppressing thyrotropin release from the bullfrog pituitary. *General and Comparative Endocrinology*, 155, pp. 574-580.
- Kang, I.H., Kim, H.S., Shin, J.H., Kim, T.S., Moon, H.J., Kim, I.Y., Choi, K.S., Kil, K.S., Park, Y.I., Dong, M.S., & Han, S.Y. (2004). Comparison of anti-androgenic activity of flutamide, vinclozolin, procymidone, linuron, and p,p'-DDE in rodent 10-day Hershberger assay. *Toxicology*, 199, pp. 145-159.
- Kannan, K., Guruge, K.S., Thomas, N.J., Tanabe, S., & Giesy, J.P. (1998). Butyltin residues in southern sea otters (*Enhydra lutris nereis*) found dead along California coastal waters. *Environmental Science and Technology*, 32, pp. 1169-1175.
- Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G.,

- Sheehan, D.M., Sinks, T., & Tilson, H.A. (1996). Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the US EPA-sponsored workshop. *Environmental Health Perspectives*, 104, pp. 715-740.
- Kavlock, R., Cummings, A. (2005). Mode of action: Inhibition of androgen receptor function - Vinclozolin-induced malformations in reproductive development. *Critical Reviews in Toxicology*, 35, pp. 721-726.
- Kelce, W.R., Lambright, L.R., Gray, L.E., & Roberts, K.P. (1997). Vinclozolin and p,p'-DDE alter androgen-dependent gene expression: In vivo confirmation of an androgen receptor-mediated mechanism. *Toxicology and Applied Pharmacology*, 142, pp. 192-200.
- Lemos, M.F.L., van Gestel, C.A.M., & Soares, A.M.V.M. (2009). Endocrine disruption in a terrestrial isopod under exposure to bisphenol A and vinclozolin. *Journal of Soils and Sediments*, 9, pp. 492-500.
- Lemos, M.F.L., van Gestel, C.A.M., & Soares, A.M.V.M. (2010a). Developmental toxicity of the endocrine disrupters bisphenol A and vinclozolin in a terrestrial isopod. *Archives of Environmental Contamination and Toxicology*, 59, pp. 274-281.
- Lemos, M.F.L., van Gestel, & C.A.M., Soares, A.M.V.M. (2010b). Reproductive toxicity of the endocrine disrupters vinclozolin and bisphenol A in the terrestrial isopod *Porcellio scaber* (Latreille, 1804). *Chemosphere*, 78, pp. 907-913.
- Lemos, M.F.L., Esteves, A.C., Samyn, B., Timperman, I., van Beeumen, J., Correia, A., van Gestel, C.A.M., & Soares, A.M.V.M. (2010c). Protein differential expression induced by endocrine disrupting compounds in a terrestrial isopod. *Chemosphere*, 79, pp. 570-576.
- Matthiessen, P., Gibbs, P.E. (1998). Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in molluscs. *Environmental Toxicology and Chemistry*, 17, pp. 37-43.
- McLachlan, J.A. (2001). Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals. *Endocrinology Reviews*, 22, pp. 319-341.
- Michel, P., Averty, B., Andral, B., Chiffolleau, J.F., & Galgani, F. (2001). Tributyltin along the coasts of Corsica (Western Mediterranean): a persistent problem. *Marine Pollution Bulletin*, 42, pp. 1128-1132.
- Mimura, H., Sato, R., Furuyama, Y., Taniike, A., Yagi, M., Yoshida, K., & Kitamura, A. (2008). Adsorption of tributyltin by tributyltin resistant marine *Pseudoalteromonas* sp. cells. *Marine Pollution Bulletin*, 57, pp. 877-82.
- Navas, J. M., Segner, H. (2006). Vitellogenin synthesis in primary cultures of fish liver cells as endpoint for in vitro screening of the (anti)estrogenic activity of chemical substances. *Aquatic Toxicology*, 80, pp. 1-22.
- Nilsson, E.E., Anway, M.D., Stanfield, J., & Skinner, M.K. (2008). Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. *Reproduction*, 135, pp. 713-721.
- Oehlmann, J., Schulte-Oehlmann, U. (2003). Endocrine disruption in invertebrates. *Pure and Applied Chemistry*, 75, pp. 2207-2218.
- Pinder, L.C.V., Pottinger, T.G. (1998). Endocrine Function in Aquatic Invertebrates and Evidence for Disruption by Environmental Pollutants. Draft report to the United

- Kingdom Environmental Agency and the CEFIC Endocrine Modulator Steering Group, 178p.
- Porte, C., Janer, G., Lorusso, L.C., Ortiz-Zarragoitia, M., Cajaraville, M.P., Fossi, M.C., & Canesi, L. (2006). Endocrine disruptors in marine organisms: Approaches and perspectives. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 143, pp. 303-315.
- Price, T.M., Murphy, S.K., & Younglai, E.V. (2007). Perspectives: The possible influence of assisted reproductive technologies on transgenerational reproductive effects of environmental endocrine disruptors. *Toxicological Sciences*, 96, pp. 218-226.
- Readman, J.W., Albanis, T.A., Barcelo, D., Galassi, S., Tronczynski, J., & Gabrielides, G.P. (1997). Fungicide contamination of Mediterranean estuarine waters: Results from a MED POL pilot survey. *Marine Pollution Bulletin*, 34, pp. 259-263
- Reddy, P.S., Sarojini, R., & Nagabhushanam, R. (1991). Impact of tributyltin oxide (TBTO) on limb regeneration of the prawn, *Caridina rajadhari*, after exposure to different time intervals of amputation. *Journal of Tissue Research*, 1, pp. 35-39.
- Rodriguez, E.M., Medesani, D.A., & Fingerman, M. (2007). Endocrine disruption in crustaceans due to pollutants: A review. *Comparative Biochemistry and Physiology A - Molecular and Integrative Physiology*, 146, pp. 661-671.
- Ruppert, E.E., Fox, R.S., & Barnes, R.D. (Eds.). (2003). *Invertebrate Zoology: A Functional Evolutionary Approach*. Brooks Cole Thomson. Belmont, CA, U.S..
- Santos, M.M., Ten Hallers-Tjabbes, C.C., Santos, A.M., & Vieira, N. (2002). Imposex in *Nucella lapillus*, a bioindicator for TBT contamination: re-survey along the Portuguese coast to monitor the effectiveness of EU regulation. *Journal of Sea Research*, 48, pp. 217- 223.
- Santos, M.M., Enes, P., Reis-Henriques, M., Kuballa, J., Castro, L.F.C., & Vieira, M.N. (2009). Organotin levels in seafood from Portuguese markets and the risk for consumers. *Chemosphere*, 75, pp- 661-666.
- Short, J.W., Thrower, F.P. (1987). Toxicity of tri-n-butyl-tin to chinook salmon, *Oncorhynchus tshawytscha*, adapted to seawater. *Aquaculture*, 61, pp. 193-200.
- Shulte-Oehlmann, U., Bettin, C., Fioroni, P., Oehlmann J., & Stroben, E. (1995). *Marisa cornuarietis* (Gastropoda, Prosobranchia): a potential TBT bioindicator for freshwater environments. *Ecotoxicology*, 4, pp. 372-384.
- Shulte-Oehlmann, U. (1997). *Fortpflanzungsstörungen bei süß- und brackwasserschnecken – Einfluß der umweltchemikalie tributylzinn*. Wissenschaft und Technik Verlag. Berlin, Germany.
- Sidharthan, M., Young, K.S., Woul, L.H., Soon, P.K., & Shin, H.W. (2002). TBT toxicity on the marine microalga *Nannochloropsis oculata*. *Marine Pollution Bulletin*, 45, pp. 177-180
- Smith, B.S. (1981). Tributyltin compounds induce male characteristics on female mud snails *Nassarius obsoletus = Ilyanassa obsoleta*. *Journal of Applied Toxicology*, 1, pp. 141-144.
- Sonak, S. (2009). Implications of organotins in the marine environment and their prohibition. *Journal of Environmental Management*, 90, pp. 1-3.
- Sousa, A., Ikemoto, T., Takahashi, S., Barroso, C., & Tanabe, S. (2009a). Distribution of synthetic organotins and total tin levels in *Mytilus galloprovincialis* along the Portuguese coast. *Marine Pollution Bulletin*, 58, pp. 1130-1136.

- Sousa, A., Laranjeiro, F., Takahashi, S., Tanabe, S., & Barroso, C.M. (2009b). Imposex and organotin prevalence in a European post-legislative scenario: Temporal trends from 2003 to 2008. *Chemosphere*, 77, pp. 566-573.
- Spooner, N., Gibbs, P.E., Bryan, G.W., & Goad, L.J. (1991). The effects of tributyltin upon steroid titres in the female dogwhelk, *Nucella lapillus*, and the development of imposex. *Marine Environmental Research*, 32, pp. 37-49.
- Szeto, S.Y., Burlinson, N.E., Rahe, J.E., Oloffs, P.C. (1989). Persistence of the fungicide vinclozolin on pea leaves under laboratory conditions. *Journal of Agricultural and Food Chemistry*, 37, pp. 529-534.
- Tabuchi, M., Veldhoen, A., Dangerfield, N., Jeffries, S., Helbing, C.C., & Ross, P.S. (2006). PCB-related alteration of thyroid hormones and thyroid hormone receptor gene expression in free-ranging Harbor seals (*Phoca vitulina*). *Environmental Health Perspectives*, 114, pp. 1024-1031.
- Takeda N. (1980). Hormonal control of head-wart development in the snail *Euchadra peliomphala*. *Journal of Embryology and Experimental Morphology*, 60, pp. 57-69.
- Takeuchi, I., Takahashi, S., Tanabe, S., & Miyazaki, N. (2004). Butyltin concentrations along the Japanese coast from 1997 to 1999 monitored by *Caprella spp.* (Crustacea: Amphipoda). *Marine Environmental Research*, 57, pp. 397-414.
- Tillmann, M., Schulte-Oehlmann, U., Duft, M., Markert, B., & Oehlmann, J. (2001). Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part III: Cyproterone acetate and vinclozolin as antiandrogens. *Ecotoxicology*, 10, pp. 373-388.
- Thomas, K.V. (2001). Antifouling paint booster biocides in the UK coastal environment and potential risks of biological effects. *Marine Pollution Bulletin*, 42, pp. 677-688
- Tomlin, C.D.S. (ed). (2003). *The pesticide manual: a world compendium*. British Crop Protection Council. Farnham, U.K.
- Ueoka, M., Allinson, G., Kelsall, Y., Graymore, M., & Stagnitti, F. (1997). Environmental fate of pesticides used in Australian viticulture: Behaviour of dithianon and vinclozolin in the soils of the South Australian Riverland. *Chemosphere*, 35, pp. 2915-2924.
- Unger, M.A., MacIntyre, W.G., & Huggett, R.J. (1988). Sorption behavior of tributyltin on estuarine and freshwater sediments. *Environmental Toxicology and Chemistry*, 7, pp. 907-915.
- U.S. EPA (1991). *Pesticide environmental fate one liner summaries: vinclozolin*. Environmental Fate and Effects Division, Washington, DC, U.S.
- U.S. EPA (1998). *Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC): final report*. Environmental Protection Agency, Washington, DC, U.S.
- U.S. EPA (2000). *Reregistration eligibility decision for vinclozolin*. Office for prevention, pesticides and toxic substances, Washington, DC, U.S.
- Vinggaard, A.M., Joergensen, E.C.B., & Larsen, J.C. (1999). Rapid and sensitive reporter gene assays for detection of antiandrogenic and estrogenic effects of environmental chemicals. *Toxicology and Applied Pharmacology*, 155, pp. 150-160.
- Waite, M. E., Evans, K. E., Thain, J. E., & Waldock, M. J. (1989). Organotin concentrations in the Rivers Bure and Yare, Norfolk Broads, England. *Applied. Organometal. Chemistry*, 3, pp. 383-391.

- Waldock, M.J., Thain, J.E. (1983). Shell thickening in *Crassostrea gigas*: Organotin antifouling or sediment induced? *Marine Pollution Bulletin*, 14, pp. 411-415
- Weis, J.S, Gottlieb, J., & Kwiatkowski, J. (1987). Tributyltin retards regeneration and produces deformities in the limbs in the fiddler crab, *Uca pugilator*. *Archives of Environmental Contamination and Toxicology*, 16, pp. 321-326



Fungicides - Beneficial and Harmful Aspects

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Fungicides are a class of pesticides used for killing or inhibiting the growth of fungus. They are extensively used in pharmaceutical industry, agriculture, in protection of seed during storage and in preventing the growth of fungi that produce toxins. Hence, fungicides production is constantly increasing as a result of their great importance to agriculture. Some fungicides affect humans and beneficial microorganisms including insects, birds and fish thus public concern about their effects is increasing day by day. In order to enrich the knowledge on beneficial and adverse effects of fungicides this book encompasses various aspects of the fungicides including fungicide resistance, mode of action, management fungal pathogens and defense mechanisms, ill effects of fungicides interfering the endocrine system, combined application of various fungicides and the need of GRAS (generally recognized as safe) fungicides. This volume will be useful source of information on fungicides for post graduate students, researchers, agriculturists, environmentalists and decision makers.

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