

## Topic 4.2

# Endocrine disruption in invertebrates\*

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*Abstract:* Recent reports have shown that a number of xenobiotics in the environment are capable of interfering with the normal endocrine function in a variety of animals. The overwhelming majority of the studies on the effects of hormone-mimetic industrial chemicals were focused on findings in vertebrates. More detailed information about the effects on and mechanisms of action in invertebrates has only been obtained from a few cases, although invertebrates represent more than 95 % of the known species in the animal kingdom and are extremely important with regard to ecosystem structure and function. The limited number of examples for endocrine disruption (ED) in invertebrates is partially due to the fact that their hormonal systems are rather poorly understood in comparison with vertebrates. Deleterious endocrine changes following an exposure to certain compounds may easily be missed or simply be unmeasurable at present, even though a number of studies show that endocrine disruption has probably occurred. The well-documented case studies of tributyltin effects in mollusks and of insect growth regulators, the latter as purposely synthesized endocrine disruptors, are explained to support this view. According to our present knowledge, there is no reason to suppose that such far-reaching changes are in any sense unique. The additional existing evidence for ED in invertebrates from laboratory and field studies are summarized as an update and amendment of the EDIETA report from 1998. Finally, conclusions about the scale and implications of the observed effects are drawn and further research needs are defined.

## INTRODUCTION

The hormonal regulation of biological functions is a common characteristic for all animal phyla, including invertebrates. While the basic endocrine strategy to regulate biological processes has been widely conserved [1], specific components of the endocrine system used in the various systematic groups have undergone significant evolutionary divergence resulting in distinct differences between the various biological taxa. This is especially true for invertebrates exhibiting a wide range of different chemical signaling systems, with some of them being unique to specific phyla. Other invertebrate groups seem to use at least partially (e.g., prosobranch mollusks) or totally (e.g., echinoderms) comparable hormones to vertebrates so that vertebrate-type sex steroids are produced in these groups and play a functional role [2–4]. Nevertheless, firm evidence of the role of these steroids in the endocrine system of invertebrates is still lacking for most phyla [5].

The endocrine systems of invertebrates generally regulate the same processes that are found in vertebrates such as development, growth, and reproduction. Because invertebrate species have devel-

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oped a huge diversity of life histories with characteristic events such as the formation of larval forms, often with a succession of different stages and/or pupation, metamorphosis, diapause or other types of resting stages, which do not occur in vertebrates, it is evident that endocrine systems of invertebrates are considerably more diverse than those found in vertebrates [2].

As it is virtually impossible to provide a complete overview of the various endocrine systems in invertebrates within the scope of this publication, we will provide a rough outline of generalities. For detailed information the reader is referred to a number of excellent reviews on invertebrate endocrinology [2,5–7]. The best-characterized invertebrate hormonal system is that of insects reflecting their economic and ecological significance and especially the need to control insect pests. Much less is known about a number of economically important aquatic groups such as crustaceans and mollusks, and knowledge on the remaining taxa is even more fragmentary.

In general, endocrine systems of invertebrates have not been documented in the same detail as vertebrates, nor have responses of invertebrate endocrine systems to suspected endocrine active substances (EASs) been studied with comparable intensity. Nevertheless, chemicals have been purposely synthesized to disrupt the endocrine system of a number of insects to aid their control. These so-called insect growth regulators (IGRs) were developed to intentionally interact with the hormonal system of these arthropods, acting as ecdysone agonists, antagonists, or juvenile hormone analogs. Perhaps one of the best-documented examples of the occurrence of EASs in the field is provided by tributyltin- (TBT-) induced imposex and intersex in gastropods [8]. The limited number of examples of endocrine disruption (ED) in invertebrates is partially due to the fact that their hormonal systems are poorly understood compared with vertebrates. Endocrine changes following an exposure to certain compounds may, therefore, be missed or simply be unmeasurable, even though some examples illustrate that invertebrates are susceptible to ED. 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 the invertebrates [8]. Studies on ED in invertebrates are important because invertebrates represent not only more than 95 % of the known species in the animal kingdom, but also provide key species for ecosystem functioning and represent an insufficiently characterized although extremely important part of global biodiversity.

The article will summarize the existing evidence for ED in invertebrates from laboratory studies and field investigations. Because this objective was also covered by the EDIETA workshop some years ago [2], we will update and amend the information provided in the workshop proceedings.

## **INVERTEBRATE ENDOCRINE SYSTEMS AND THEIR SUSCEPTIBILITY TO ENDOCRINE-DISRUPTING COMPOUNDS**

Any multicellular organism requires coordinated mechanisms that, besides direct cell contacts, involve chemical messengers. Consequently, all known invertebrate taxa make use of hormones to control biochemical, physiological, and behavioral processes in general as well as development, growth, and reproduction in particular. Because they are represented by more than 30 different phyla within the animal kingdom, it is not surprising that regulation of the above-mentioned processes by their endocrine systems is considerably more diverse than in vertebrates, which comprise only part of a single phylum, the Chordata. Despite the diversity in invertebrate endocrinology, some basic generalities can be made. Invertebrates use steroids, terpenoids, and peptide hormones, but the latter are by far the most common among these phyla [3,9]. While steroids are secreted in vertebrates from true glands, the secretory structures in invertebrates are often neuronal in origin and therefore referred to as neurosecretory organs or cells. Steroids, such as ecdysone and the vertebrate-type steroids, differ from terpenoid and especially peptide hormones in their physical and chemical properties, solubility, and resistance to degradation. A further issue that has to be emphasized is that certain compounds are likely to act as endocrine disruptors not only by a direct binding to receptors—acting as hormone-mimics (agonists) or as “anti-hormones” (antagonists)—but also indirectly by modulating endogenous hormone levels by interfering

with biochemical processes associated with the production, availability, or metabolism of hormones or also by the modulation of receptors. Therefore, it is likely that the various endocrine systems in invertebrates are subject to modulation by an unforeseeable number of exogenous compounds.

Table 1 summarizes the occurrence of major hormone groups in various invertebrate taxa, based on a number of reviews on invertebrate endocrinology [2,5–7], but without intending to provide a complete list. The majority of invertebrates are not considered in the table because the endocrinology of these organisms remains largely unknown. It is obvious that the perhaps best-understood endocrine systems are those of insects, followed by crustaceans, echinoderms, and mollusks, although the latter are perhaps the most diverse of the invertebrate phyla, being second to the insects in number of identified species. The endocrine systems of the various classes of mollusks and even of major groups of gastropods—prosobranchs, opisthobranchs, and pulmonates—differ greatly, reflecting extreme differences in morphology and life histories. This can be exemplified by the vertebrate-type steroids, which do occur in prosobranchs and play a functional role. In contrast, there is no indication that opisthobranchs and pulmonates use steroids. Recent, still unpublished studies in Japan, Germany, and the United Kingdom demonstrated the occurrence of estrogen and androgen receptors in a number of marine and freshwater prosobranchs and characterized the receptors with regard to ligand-binding and structure (e.g., for *Thais clavigera*).

**Table 1** Examples of reported hormones in different invertebrate taxa [2,5–7].

Taxon	Reported hormones (example, <i>controlled process</i> )
Coelenterata	Neuropeptides (glycine-leucine tryptophan amides = GLWamides, <i>metamorphosis</i> ); thyroids (thyroxine, <i>strobilation</i> ); retinoids (9- <i>cis</i> -retinoic acid, <i>strobilation</i> )
Nematoda	Ecdysteroids (reported but <i>functional role questionable</i> ); terpenoids [juvenile hormone (JH) like hormones, <i>growth</i> ]; neuropeptides (FMRFamide, <i>function unknown</i> )
Mollusca	Ecdysteroids (reported but <i>role questionable</i> ); steroids (17 $\beta$ -estradiol, testosterone, progesterone, <i>sexual differentiation, reproduction in prosobranchs</i> ); terpenoids (JH reported but <i>role questionable</i> ); neuropeptides [APGWamide, dorsal body hormone (DBH), <i>sexual differentiation, gonad maturation, spawning</i> ; egg-laying hormone (ELH), <i>spawning</i> ; FMRFamide, <i>neuromodulation</i> ; molluscan insulin-like peptides (MIPs), <i>growth, development, energy metabolism</i> ]
Annelida	Ecdysteroids (ecdysone, <i>role unknown</i> ); neuropeptides (FMRFamide, <i>neuromodulation</i> )
Crustacea	Ecdysteroids (ecdysone, <i>molting, vitellogenesis</i> ); steroids (17 $\beta$ -estradiol, testosterone, progesterone, <i>role under debate</i> ); terpenoids [methyl farnesoate (MF), <i>metamorphosis, reproduction</i> ]; neuropeptides [androgenic hormone, <i>sexual differentiation, vitellogenesis inhibition</i> ; crustacean hyperglycemic hormone family (CHH), <i>energy metabolism</i> ; molt-inhibiting hormone (MIH), <i>ecdysteroid production</i> ; vitellogenesis-inhibiting hormone (VIH), <i>vitellogenesis</i> ]
Insecta	Ecdysteroids (ecdysone, <i>molting, egg maturation</i> ); terpenoids (JH, <i>metamorphosis, reproduction</i> ); neuropeptides [adipokinetic hormone (AKH), <i>energy metabolism</i> ; allatostatin and allatotropin, <i>JH production</i> ; bombyxin, <i>ecdysteroid production, energy metabolism</i> ; bursicon, <i>cuticle tanning</i> ; diapause hormone, <i>embryonic diapause</i> ; diuretic hormone (DH), <i>water homeostasis</i> ; ecdysis-triggering hormone (ETH) and eclosion hormone (EH), <i>ecdysis behavior</i> ; FMRFamides, <i>neuromodulation</i> ; prothoraciotrophic hormone (PTTH), <i>ecdysteroid production</i> ]
Echinodermata	Steroids (progesterone, testosterone, 17 $\beta$ -estradiol, estrone, <i>vitellogenesis, oogenesis, spermatogenesis, spawning</i> ); neuropeptides (gonad-stimulating substance = GSS, <i>spawning</i> ; maturation-promoting factor = MPF, <i>fertilization</i> )
Tunicata	Steroids (testosterone, 17 $\beta$ -estradiol, <i>oogenesis, spermatogenesis, spawning</i> ); neuropeptides (gonadotropin releasing hormone analogue, <i>gonad development</i> ); thyroids (thyroxine, <i>probably tanning process during tunic formation</i> )

## EVIDENCE FOR ENDOCRINE DISRUPTION IN INVERTEBRATES

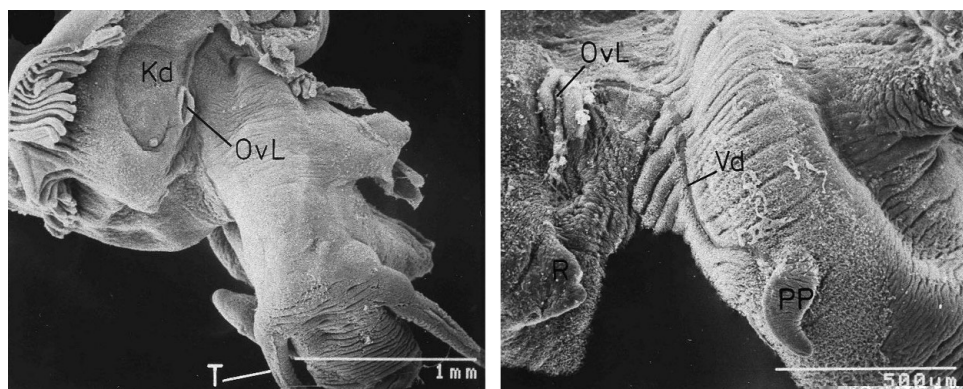
The issue of ED in invertebrates has found an increasing scientific interest although only a limited number of confirmed cases have been reported. These are dominated by the antifouling biocide tributyltin (TBT) and its effects on prosobranch snails and by IGRs which were designed as EASs for use in insect pest control. The following sections will provide a summary of these confirmed examples of ED and an update of the detailed synopsis from the EDIETA workshop [2].

### Organotin compounds and their effects in mollusks

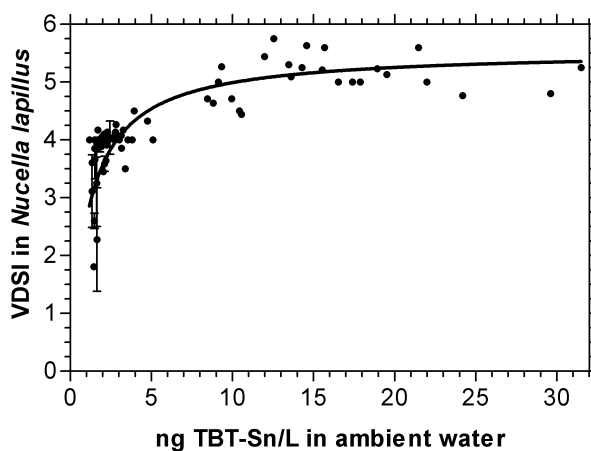
The effects of TBT on prosobranch snails are one of the most complete examples of an EAS impact on aquatic invertebrates [8]. TBT compounds are mainly used as biocides in antifouling paints, but also in other formulations. They induce a variety of malformations in aquatic animals with mollusks as one of the most TBT-sensitive groups [10]. As the impact of TBT on nontarget organisms became apparent in the early 1980s, France drew up regulations to control TBT emission and banned the use of TBT antifouling on small boats (length <25 m) in 1982, adopted later by other countries since 1987. Nevertheless, TBT pollution of coastal waters was found to have remained on a high level or even increased further in some regions. Consequently, the International Maritime Organization (IMO) decided in autumn 2001 to ban the application of TBT-based paints on all boats by January 2003 and the presence on ship hulls by January 2008.

The first adverse effects of TBT on mollusks were observed in *Crassostrea gigas* at the Bay of Arcachon, one of the European centers of oyster aquaculture, with ball-shaped shell deformations in adults and a decline of annual spatfall [11]. These effects led to a break-down of local oyster production with marked economic consequences. Laboratory and field analyses revealed that TBT was the causative agent with trace concentrations as low as 10 ng TBT/L in ambient water being effective [10].

Shell deformities in oysters, but also in other bivalves, were found in the Bay of Arcachon in 1982. The deformities were characterized by a ball-shaped shell deformation in adults and a decline of annual spatfall [11]. These effects led to a break-down of local oyster production with marked economic consequences. Laboratory and field analyses revealed that TBT was the causative agent with trace concentrations as low as 10 ng TBT/L in ambient water being effective [10].



**Fig. 1** *Hydrobia ulvae*. Scanning electron micrographs of females with opened mantle cavity. Left: female without imposex; right: sterilized imposex female with blocked oviduct. Abbreviations: Kd, capsule gland; OvL, ooporous oviduct opening (open left; blocked right); PP, penis; T, tentacle; Vd vas deferens.



**Fig. 2** *Nucella lapillus*. Relationship between aqueous TBT concentrations and imposex intensities:  $y = (5.54 x) / (1.12 + x)$ ;  $n = 151$  population samples from 81 stations;  $r = 0.688$ ;  $p < 0.0005$ .

The periwinkle *Littorina littorea* develops a closely related TBT-induced virilization phenomenon, termed as intersex [17]. Intersex-affected females exhibit male features on female pallial organs (inhibition of the ontogenetic closure of the pallial oviduct), or female sex organs are supplanted by the corresponding male formations. Comparably to imposex, the intersex response is a gradual transformation of the female pallial tract, which can be described by an evolutive scheme with four stages [17]. Intersex development restricts the reproductive capability of females. In stage 1, sperm are lost during copulation, and consequently the reproductive success is reduced. Females in stages 2–4 are definitively sterile because the capsular material is spilled into the mantle cavity (stage 2) or the glands responsible for the formation of egg capsules are missing (stages 3 and 4). Due to female sterility, periwinkle populations can be in decline, but are not likely to become extinct because of the planktonic veliger larvae, as long as aqueous TBT levels are not beyond mortality threshold concentrations for the larvae [18].

The assessment of intersex intensities in periwinkle populations is based on the same principle as described for the VDSI. The intersex index (ISI) is the mean intersex stage in a population. ISI values are highly significantly correlated to ambient TBT concentrations and can therefore be used together with or as an alternative to imposex assessments for the determination of the degree of coastal TBT pol-

lution especially in regions with a relatively high level of contamination. In these areas, periwinkles are very common and can be sampled in sufficient numbers because *L. littorea* (a) is more tolerant of high TBT levels, (b) recruits from the plankton, and (c) can occur in areas where dog whelks have expired. Imposex in dog whelks and intersex in periwinkles have been used as combined biological markers for the convention-wide biological TBT effect monitoring of OSPAR (Oslo and Paris Commissions) [19].

Furthermore, TBT affects the community level and exhibits negative effects even beyond the scale of populations. Waldo et al. [20] analyzed the inter- and subtidal fauna of the River Crouch in a number of subsequent surveys between 1987 and 1992 and compared the results with older reports before the introduction of TBT-based antifouling paints. Overall, directional trends in community level attributed at a number of analyzed stations suggested a moderate improvement in environmental conditions over the sampling period, which was coincident with a marked decline in TBT concentrations at the stations. However, reference to historical data indicated that certain taxa that were previously frequent or common, especially snails, were only rarely recorded or still absent in the 1992 survey.

Much less attention has been paid to endocrine effects of TBT in freshwater ecosystems. The ramshorn snail *Marisa cornuarietis* and the hydrobiid *Potamopyrgus antipodarum* exhibit endocrine-mediated effects of TBT [21]. The latter and the netted whelk *Nassarius reticulatus* were used for a monitoring of androgenic activities in sediments of the River Elbe [22]. The majority of sediments exhibited marked androgenic activities and some of them, assigned to the ecological status classes IV and V according to the European Water Framework Directive, caused a maximum increase of imposex intensities in the netted whelk within four weeks.

At the molecular level, TBT interferes with hormone metabolism, most probably by an inhibition of the cytochrome P450-dependent aromatase, increasing the levels of androgens [8,23,24], while findings from the early 1980s showed that TBT inhibits the release of a neuroendocrine factor from the pleural ganglia, which is responsible for the suppression of penis formation in females, thus resulting in imposex development [25]. Although the factor has not been identified, recent results show that an administration of the neurohormone APGWamide can induce imposex in *Ilyanassa obsoleta* [26]. A possible explanation for these conflicting findings is the hypothesis of an even more pronounced analogy of vertebrate and prosobranch hormonal systems with neurohormones acting as releasing factors in both systematic groups, mediating steroid production and/or metabolism [27].

It has been accepted that imposex is induced almost typically by TBT [2], although at least in the marine rock shell *Thais clavigera* [28] and the freshwater ramshorn snail *Marisa cornuarietis* [29] not only TBT, but also triphenyltin (TPT) can promote imposex, while in other prosobranchs, such as *Nucella lapillus* and *Nassarius reticulatus*, TPT does not induce imposex [29].

One of the most important lessons to be learned from TBT and its effects in mollusks is that EASs may impact different levels of biological integration from molecules to communities affecting also the survival of populations in the field. Furthermore, the case history of TBT provides evidence that vertebrate-type steroids play an important functional role in a number of invertebrate groups, including prosobranchs.

### **Insect growth regulators (IGRs) as purposely synthesized EASs**

The IGRs were developed to intentionally mimic, block, or otherwise interact with the hormonal system of insects, so that it is not too astonishing that they represent—next to TBT—a second group of xenobiotics which was rated by the EDIETA workshop as a confirmed case for ED. The workshop report [2] summarizes not less than 47 references, mainly laboratory studies but also a number of field investigations with a focus of IGR effects on nontarget species. Most of these studies were conducted with terrestrial species, while possible effects on aquatic insects, e.g., from IGR spray drift or run-off during or after agricultural application received only a little attention.

Ecdysteroid receptor agonists such as tebufenozide, methoxyfenozide, or RH 5849 induce symptoms of hyperecdysionism in terrestrial insects, delayed postembryonic development, and nymphal-adult

intermediates. Tebufenozide interacts with the ecdysone receptor complex. Paradoxically, even though most insects and other arthropods use ecdysone as a molting hormone, tebufenozide is selectively toxic to the Lepidoptera [30]. Ecdysteroid antagonists such as azadirachtin or KK-42 prevent normal diapause induction, and induce an early termination of diapause or a precocious metamorphosis, while juvenile hormone (JH) analogs, such as fenoxycarb, methoprene, and pyriproxyfen, interfere with egg hatching, larval development, larval-pupal molts, and ecdysis and reduce the fertility and longevity of exposed specimens.

Despite these marked effects in insects, there are major terrestrial invertebrate classes for which there are no apparent data on the impact of IGRs, e.g., earthworms and mollusks. There are also no reported incidents of effects on terrestrial invertebrates of nonpesticidal endocrine disruptors that are present in the environment.

Occasionally, chitin synthesis inhibitors (CSIs), such as diflubenzuron, other benzylphenyl ureas, and related compounds [31] have been misreported as EASs. Although they interfere with molting, an endocrine-regulated process in arthropods, the mechanism of CSI action is purely nonendocrine. CSIs inhibit one of the steps of chitin synthesis selectively. Because this synthesis usually takes place at or during the time of molting, CSIs cause death during the molt, resembling the effects of endocrine-mimicking IGRs, albeit not via an endocrine pathway.

### Further cases of endocrine disruption in aquatic and terrestrial invertebrates

Next to the effects of TBT in prosobranchs and of IGRs in insects, a number of further laboratory and field studies have been reported, where compounds exhibited effects on endocrine-regulated processes in marine, freshwater, and terrestrial invertebrates. For the period until late 1998, the EDIETA report [2] summarizes not less than 56 studies in which ED may have occurred although nonendocrine mechanisms are also possible for the observed effects (Table 2).

**Table 2** Synopsis of studies, reported in [2], in which ED may have occurred, although nonendocrine mechanisms are also possible for the observed effects (f, freshwater; m, marine; t, terrestrial; tot, total).

Phylum	Contaminants	Effects	Cited studies
Mollusca	Cd, DDT, MCPA (2-methyl-4-chlorophenoxyacetic acid)	Fecundity alterations; spawning stimulation; inhibition of gonadal development	f: 1; m: 1; t: - <b>tot: 2</b>
Annelida	Volatile organic compounds from crude oil	Induction of spawning	f: -; m: 1; t: - <b>tot: 1</b>
Crustacea	Atrazine, Cd, DES (diethylstilbestrol), diazinon, dieldrine, diurone, endosulfane, Hg, lindane, methoprene, naphthalene, NP (nonylphenol), OP (octylphenol), PB, PCBs, PCP (pentachlorophenole), phthalate esters, Se, sewage outfall, simazine, TBT, Zn	Elevated ecdysteroids levels; interference with molt, growth, energy metabolism and fecundity; delayed maturity; mortality; increased intermolt duration; inhibition of larval development; abnormal coloration; disrupted testosterone metabolism; induction of cyprid major protein; intersexuality; retarded limb regeneration and limb abnormalities	f: 24; m: 11; t: - <b>tot: 35</b>
Insecta	NP, phthalate esters, metal containing effluents, PAHs, tannery and paper mill effluent, various organic and inorganic pollutants, Zn	Mortality; various mouthpart deformities; other pathomorphological changes; - interference with molt cycle and frequency	f: 6; m: -; t: 2 <b>tot: 8</b>
Echinodermata	Cd, estradiol, estrone, PCBs, PCP, Zn	Abnormal embryogenesis and development; low fertilization success; reduced ovarian growth; elevated or reduced steroids levels; increased oocyte growth	f: -; m: 10; t: - <b>tot: 10</b>

The crustaceans represent the systematic group providing the majority of cases of suspected ED shown in Table 2. While the examples for the aquatic environment are almost balanced between fresh-water and marine species, only two studies report comparable effects in terrestrial arthropods.

Since the publication of the EDIETA report, not less than 25 new cases for ED in invertebrates have been published. These are summarized in Table 3 but without the intention of providing a complete list, because unpublished data from currently ongoing research programs for the identification of new suitable test species and sensitive endpoints could not be considered.

**Table 3** Additional laboratory and field studies with evidence for ED, which have been published since the EDIETA workshop [2]. Abbreviations: LCT, life cycle test.

Phylum	Species (life stage)	Contaminant (conc. range)	Effects observed	Lab/ field	Ref.
Porifera	<i>Heteromyenia</i> sp., <i>Eunapius fragilis</i> (gemmulae - adults)	Bisphenol A (BPA) (0.16–160 mg/l), ethylbenzene (0.03–3 mg/l), NP (0.022–22 mg/l)	Reduced growth rates; developmental abnormalities with malformed water vascular systems (especially at lower concentrations)	Lab	[46]
Rotatoria	<i>Brachionus calyciflorus</i> (adults)	Flutamide, NP, testosterone (1–50 µg/l for all)	Fertilization of females inhibited	Lab	[55]
Mollusca	<i>Marisa cornuarietis</i> (adults, LCT), <i>Potamopyrgus antipodarium</i> , <i>Nucella lapillus</i> , <i>Nassarius reticulatus</i> (adults)	BPA (0.05–100 µg/l), OP (1–100 µg/l)	Induction of “superfemales”: stimulation of egg/embryo and spawning mass production, additional female sex organs ( <i>Marisa</i> ), oviduct malformations and increased female mortality ( <i>Marisa</i> )	Lab	[32,52]
	<i>M. cornuarietis</i> , <i>N. lapillus</i> , <i>N. reticulatus</i> (adults)	TPT (0.005–0.5 µg as Sn/l)	Imposex development ( <i>Marisa</i> ); reduction of female sex glands; impairment of spermatogenesis and oogenesis	Lab	[29]
	<i>M. cornuarietis</i> , <i>N. lapillus</i> , <i>N. reticulatus</i> (adults)	Cyproterone acetate (1.25 mg/l), vinclozolin (0.03–1 µg/l)	Suppression of imposex development from TBT; reduction of male sex glands and penis; advancement of sexual repose	Lab	[33]
	<i>Mya arenaria</i> (adults)	Estradiol, NP, PCP, contaminated natural sea water	Induction of vitellogenin-like proteins by test chemicals; reduced levels in the field (due to (anti-)estrogens?)	Lab & field	[37]
	<i>M. arenaria</i> (adults)	Unknown (field survey) NP	Delayed gametogenesis; dysfunction of vitellogenesis	Field	[41]
	<i>Crassostrea gigas</i> (larvae)	(0.1–10000 µg/l)	Delayed development to D-stage; reduced survival; malformed D-larvae	Lab	[50]
Annelida	<i>Dinophilus gyro-ciliatus</i> (adults)	NP	Stimulation of egg production; reduced egg viability	Lab	[38]
Crustacea	<i>Daphnia magna</i> (LCT)	Ponasterone A (3.4–27 nM)	Reduced fecundity in F <sub>2</sub> generation; incomplete ecdysis; premature death	Lab	[34]
	<i>D. magna</i> (adults)	Cyproterone acetate (0.3–5 µM)	Molt-independent growth reduction; reduced offspring numbers	Lab	[48]
	<i>D. magna</i> (adults)	Androstenedione (6.2–25 µM), DES (0.75–3 µM), methoprene (0.08–0.32 µM)	Stimulation of abdominal process by DES and methoprene and of development of first antennae by androstenedione	Lab	[53]
	<i>Gammarus pulex</i> (adults)	Unknown (field survey)	Abnormal oocyte structure during vitellogenesis; reduced length and male/female size differences	Field	[42]

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Table 3 (Continued).

Phylum	Species (life stage)	Contaminant (conc. range)	Effects observed	Lab/ field	Ref.
	<i>Corophium volu- tator</i> (juv. – adults)	NP (>10 µg/l)	Mortality; reduced growth; increased female fertility and antennae length	Lab	[39]
	<i>Balanus amphitrite</i> (larval stages)	NP (0.01–1 µg/l) estradiol (1 µg/l)	Induction of CMP (cypris major protein)	Lab	[35]
	<i>Elminius modestus</i> (larval stages)	NP (0.01–10 µg/l) estradiol (10 µg/l)	Altered timing of larval development; reduced growth	Lab	[36]
	<i>Palaemonetes pugio</i> (adults)	Pyrene	Induction of vitellin	Lab	[51]
Insecta	<i>Drosophila melano- gaster</i> (cell line)	80 different test chemicals	Receptor-mediated ecdysteroid response as screening tool	Lab	[40]
	<i>Lacania oleracea</i> (larvae to adults)	Estradiol, methyl- testosterone, thyroxine (1 mg/kg dietary dose)	Increased length and reduced weight of older larval stages; deformed pupae, reduced fecundity and egg viability (only methyltestosterone)	Lab	[47]
	<i>Chironomus riparius</i> (LCT)	Tebufenozide (1–100 µg/l)	Mortality during pupation and emergence with sex-related differences	Lab	[43]
	<i>C. riparius</i> (LCT)	TBT (0.01–5 µg as Sn/l)	Ecdysteroid synthesis (males: increased females: decreased); development (males: faster, females: slower)	Lab	[44]
	<i>C. riparius</i> (LCT)	BPA (1–3000 µg/l), NP (1.9–2000 µg/l)	Alteration of vitellogenin/vitellin production in males	Lab	[45]
	<i>C. riparius</i> (larvae)	NP (10–100 µg/l)	Increased frequency of mouthpart deformities	Lab	[49]
Tunicata	<i>Ciona intestinalis</i> (larvae)	TBT (up to 10–5 M)	Reduced thyroxine production; block of metamorphosis	Lab	[54]

It is not possible to present and discuss the publications on ED in invertebrates, summarized in Table 3, in detail, but two general observations should be emphasized: (1) Endocrine-mediated effects of xenobiotics in terrestrial species still constitute less than 10 % of all reports, and (2) although single studies of ED in formerly ignored taxa are available now (e.g., for Porifera, Rotatoria, Tunicata), the overwhelming majority of publications focus on mollusks, crustaceans, and insects, thus continuing the main tendencies in the pre-1999 literature.

## CONCLUSIONS ABOUT THE SCALE AND IMPLICATIONS OF EFFECTS

Despite the fact that an increasing number of research projects are investigating ED in invertebrates worldwide and that the published examples of potential effects of EASs in these groups has increased by almost 50 % in the last three years since the EDIETA workshop, the main conclusions are still valid [2]. With the exception of TBT effects in mollusks, which have been associated with a locally severe impact at the community level, and IGRs in terrestrial insects, there are only a few field examples of ED in invertebrates. Nevertheless, it is suspected that there are many more examples for ED affecting invertebrate populations and communities, though still undetected. This assumption is supported by the following indications: (1) The basic mechanisms of chemical signaling systems exhibit a considerable degree of conservatism throughout the animal kingdom [1] so that invertebrate endocrine function should be affected by the same or similar compounds as those of vertebrates [2,5]. (2) For purposes of pest control, a number of highly effective EASs have been intentionally developed to interfere with the hormonal systems of insects. There is no reason to suppose that such endocrine-mediating properties are unique for the IGRs, but rather reflect the fact that much less research has been undertaken for other invertebrate groups than insects. (3) ED in invertebrates has found far less attention than in vertebrates, probably because their hormonal systems are poorly understood, favoring investigations with verte-

brates and especially fish as systematic groups for ecotoxicological research and routine analyses many scientists feel familiar with.

## RESEARCH NEEDS

Our ignorance of invertebrate endocrinology is one of the main reasons for the unsatisfactory progress that has been made regarding ED in invertebrates. A further important point is that ED in vertebrates has attracted a higher degree of public and even scientific awareness, which is also reflected by funding resources and other economic circumstances making the general conditions for research with invertebrates less favorable. Nevertheless, the consideration of invertebrates in such research programs potentially offers a wealth of knowledge in understanding comparative and ecological aspects of ED [2]. For these reasons, invertebrates should have a high priority for further research, especially for the development of testing and monitoring techniques:

- More basic research on invertebrate endocrinology is needed, especially for groups that were almost totally neglected in the past (i.e., not considered in Table 1).
- Hormone receptors of invertebrates should be identified, cloned, and characterized, facilitating the identification of receptors that are shared by different groups. This would help to develop receptor-binding assays and other *in vitro* systems as a screening tool.
- Endocrine control of toxicological endpoints in tests should be characterized in more depth so that these endpoints can be used as valid measures for ED.
- New invertebrate tests with endocrine-regulated endpoints have to be developed or existing protocols amended. This will require a broad initiative with a variety of invertebrate assays. In a second step, these tests will have to be validated, including the use of reference compounds and positive controls known to have endocrine-disrupting properties in the systematic groups under investigation.
- It is important to emphasize that several sentinel species will be required since the endocrinology of invertebrates differs widely among taxa. Therefore, representatives of each phylum are needed. For the freshwater environment test and monitoring species should at least include Annelida, Mollusca, Crustacea, and Insecta, in the marine environment Coelenterata, Annelida, Mollusca, Crustacea, and Echinodermata, and an even more diverse list of taxa for terrestrial ecosystems.
- The current knowledge of valid endocrine-mediated endpoints in invertebrates is too incomplete to design specific monitoring programs for biological effects of EASs, perhaps with the exception of androgenic and estrogenic compounds and their effects in prosobranch snails. Nevertheless, it is obvious that carefully targeted monitoring programs are needed because effects in invertebrates are probably widespread but undetected.

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