Evidence for Endocrine Disruption in Invertebrates

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The issue of endocrine disruption (ED) in invertebrates has generated remarkably little interest in the past compared to research with aquatic vertebrates in this area. However, with more than 95% of all known species in the animal kingdom, invertebrates constitute a very important part of the global biodiversity with key species for the structure and function of aquatic and terrestrial ecosystems. Despite the fact that ED in invertebrates has been investigated on a smaller scale than in vertebrates, invertebrates provide some of the best documented examples for deleterious effects in wildlife populations following an exposure to endocrine-active substances. The article provides an overview of the diversity in endocrine systems of invertebrates. The principal susceptibility of invertebrates to endocrine-active compounds is demonstrated with the case studies of tributyltin effects in mollusks and of insect growth regulators, the latter as purposely synthesized endocrine disrupters. The additional evidence for ED in invertebrates from laboratory and field studies is 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 research needs are defined.

KEY WORDS: Endocrine disruption, Invertebrates, Annelids, Crustaceans, Echinoderms, Insects, Mollusks, Porifera, Rotatoria, Tunicata, Tributyltin, Insect growth regulators.

I. Introduction

All animal phyla use chemical signaling systems to regulate biological functions. Because hormonal control of developmental processes such as growth, sexual differentiation, and reproduction is such a widespread and

common characteristic, it is not too surprising that invertebrates also use hormones. While the basic endocrine strategy to regulate biological processes has been widely conserved (McLachlan, 2001), specific components of the endocrine system used in various systematic groups have undergone significant evolutionary divergence, resulting in distinct differences among various biological taxa. Especially within invertebrates a huge diversity of chemical signaling systems can be found and some of the hormones used are unique for specific phyla. Other invertebrate groups such as prosobranch mollusks and echinoderms use at least partially or even totally comparable hormones as vertebrates so that vertebrate-type sex steroids are produced in these groups and play a functional role (deFur *et al.*, 1999; Hines *et al.*, 1992; Lafont, 2000). Nevertheless, firm evidence of the role of these steroids in the endocrine system of invertebrates is still lacking for most phyla (Pinder and Pottinger, 1999).

Generally, endocrine systems of invertebrates regulate developmental processes identical to those found in vertebrates, including growth, sexual differentiation, and reproduction. Invertebrate species have developed a considerable 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. Consequently, it is evident that endocrine systems of invertebrates are considerably more diverse than those found in vertebrates (deFur *et al.*, 1999).

It is almost impossible to provide a comprehensive overview of the various endocrine systems of invertebrates here. Therefore, a rough outline of generalities is provided. For more detailed information the reader is referred to a number of excellent reviews on invertebrate endocrinology (deFur *et al.*, 1999; Dorn, 2000; Hoffmann and Porchet, 1984; Pinder and Pottinger, 1999). The endocrine system of insects is clearly the best characterized within the invertebrates reflecting their economic and ecological significance and especially the need to control insect pests. By far less is known about a number of economically important aquatic groups such as crustaceans and mollusks and the knowledge on the remaining taxa is even more fragmentary.

It is generally agreed that the hormonal systems of invertebrates are not as well documented as those of vertebrates. Furthermore, potential effects of suspected endocrine-disrupting chemicals (EDCs) on the various invertebrate endocrine systems have not been studied as well as in fish. 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) represent third-generation insecticides and 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 for the occurrence of EDCs in the field is provided by tributyltin (TBT)-induced imposex and intersex in gastropods (Matthiessen and Gibbs, 1998).

Although the case studies of IGR effects in insects and of TBT-induced imposex and intersex in prosobranch snails are the perhaps the most complete examples of endocrine disruption (ED) in wildlife populations, further examples for ED in invertebrates are rather limited. This is partially due to the fact that their hormonal systems are poorly understood compared with vertebrates. Endocrine changes following exposure to certain compounds may therefore be missed or simply be unmeasurable at present, even though a number of further examples exist indicating 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 (Matthiessen and Gibbs, 1998).

It is the main objective of this article to summarize the existing evidence for ED in invertebrates from both laboratory studies and field investigations. Because this objective was also covered by the EDIETA workshop some years ago (deFur *et al.*, 1999), the current attempt will update and amend the evidence for ED in invertebrates to August 2003.

II. Invertebrate Endocrine Systems and Their Susceptibility to Endocrine-Disrupting Compounds

Almost all metabolic processes in metazoans require coordinated mechanisms that, besides direct cell contacts, involve chemical messengers. Therefore, all known invertebrate taxa use hormones to control biochemical, physiological, and behavioral processes in general as well as development, growth, and reproduction in particular. Invertebrates represent more than 30 different phyla within the animal kingdom. Consequently, it is not surprising that regulation of the above-mentioned processes by their endocrine systems is considerably more variable than in vertebrates, which comprise only part of a single phylum, the Chordata.

Despite the fact that invertebrate hormonal systems are that diverse, 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 (Gorbman and Davey, 1991; Lafont, 2000). Although steroids are secreted in vertebrates from true glands, the secretory structures in invertebrates are often neuronal in origin and are therefore referred to as neurosecretory organs or cells. Steroids, such as ecdysone and the vertebrate-type

steroids such as 17β-estradiol and testosterone, differ from terpenoid and especially peptide hormones in their physical and chemical properties, solubility, and resistance to degradation. A further issue to be emphasized is that certain compounds are likely to act as endocrine disrupters not only by direct binding to receptors—acting as hormone mimics (agonists) or as "antihormones" (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, endocrine systems in invertebrates can be presumed to be subject to modulation by a not foreseeable number of exogenous compounds as well.

Table I summarizes the occurrence of the major hormone groups in various invertebrate taxa, based on a number of reviews on invertebrate endocrinology (deFur *et al.*, 1999; Dorn, 2000; Hoffmann and Porchet, 1984; Pinder and Pottinger, 1999). It is obvious that perhaps the best understood endocrine systems are those of insects, followed by crustaceans, echinoderms, and mollusks, although the latter are perhaps characterized by the most diverse hormonal systems of the invertebrate phyla. The endocrine systems of the various classes of mollusks and even of major groups of gastropods—prosobranchs, opisthobranchs, and pulmonates—differ considerably, reflecting extreme differences in morphology and life histories. This can be exemplified by the use of vertebrate-type steroids, which do occur and play a functional role in prosobranchs. In contrast, there is no indication for pulmonates using steroids. Recently, the first estrogen receptor sequence for an opisthobranch mollusk, the sea hare *Aplysia californica*, was published by Thornton *et al.* (2003). Further still unpublished studies in Japan, Germany and the UK demonstrate the occurrence of estrogen and androgen receptors in a number of marine and freshwater prosobranchs and characterize the receptors with regard to ligand binding and structure (e.g., for *Thais clavigera* and *Marisa cornuarietis*).

III. Evidence for Endocrine Disruption in Invertebrates

The issue of ED in invertebrates has found increasing scientific interest in the past, although only a limited number of confirmed cases were reported. These are clearly dominated by the antifouling biocide TBT and its worldwide effects on prosobranch snails and IGRs, which were designed to act as EDCs for use in insect pest control. The following sections will provide a summary of these confirmed examples for ED.

TABLE I
Examples of Reported Hormones in Different Invertebrate Taxa[a]

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

[a]From Hoffmann and Porchet (1984), deFur *et al.* (1999), Pinder and Pottinger (1999), and Dorn (2000).

A. Mollusks: Organotin Compounds and Their Effects in Gastropods and Bivalves

TBT effects in prosobranch snails are ranked among the best documented examples of the impact of an EDC on aquatic invertebrates (Matthiessen and Gibbs, 1998; Matthiessen and Law, 2002). These organotin compounds are mainly used as biocides in antifouling paints, but also in various other formulations. They produce a variety of malformations in aquatic animals with mollusks as one of the most TBT-sensitive groups of invertebrates (Bryan and Gibbs, 1991). In the early 1980s the first impact of TBT on nontarget organisms was observed in France (Alzieu *et al.*, 1980). France was the first country to draw up regulations to control TBT emission from antifouling paints and banned the use of TBT-based antifoulings on small boats (length <25 m) in 1982. This legislation was adopted later by other countries. Although a consequent drop of aqueous TBT concentrations was expected and reported for some regions, TBT pollution of coastal waters was found to have remained on a high level or even increased further in other areas. 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 on ship hulls by January 2008. Meanwhile, the proposed ban has been enacted to law in many countries worldwide.

The first adverse effects of TBT on mollusks were observed in *Crassostrea gigas* at the Bay of Arcachon, one of the centers of oyster aquaculture in Europe, with ball-shaped shell deformations in adults, and a dramatic decline of annual spatfall (Alzieu *et al.*, 1980). These effects led to a breakdown of local oyster production in the bay with marked economic consequences. Laboratory and field analyses revealed that TBT from antifouling paints was the causative agent with trace concentrations as low as 10–20 ng TBT/liter in ambient water being already effective (Bryan and Gibbs, 1991). Shell deformities in oysters as well as other bivalve groups have been used as biological markers of TBT effects (Oehlmann and Schulte-Oehlmann, 2003). Another TBT effect in mollusks was initially described in a number of regions worldwide in the early 1970s without identifying the organotin compound as the causative agent at that time: a virilization of female prosobranchs, which has been termed imposex (Smith, 1971). Imposex is characterized by the formation of a penis and/or vas deferens on females of gonochoristic prosobranch species and is induced at lower concentrations than all other described TBT effects. Furthermore, it is a specific response of organotin compounds under field conditions.

Today, imposex is known to occur in more than 150 prosobranch species. An incomplete list is provided by deFur *et al.* (1999). The gradual virilization of imposex-affected females is described by a classification scheme with six stages, further divided in up to three different types (a–c) (Fioroni *et al.*, 1991) having the advantage of being applicable to all affected species. Females are sterilized in imposex stages 5 and 6 by a blockade of the pallial oviduct (Fig. 1) or by a split bursa copulatrix and capsule gland. The first mode of functional sterilization prevents the release of egg capsules, resulting in an accumulation of abortive capsular material in the pallial oviduct; the second mechanism prevents copulation and capsule formation. In young and sexually immature specimens of some muricid species a protogyne sex change can be induced by TBT concentrations, e.g., above 10 ng as Sn/liter in *Nucella lapillus* (Gibbs *et al.*, 1987) and above 2 ng as Sn/liter in *Ocinebrina aciculata* (Oehlmann *et al.*, 1996a).

The classification scheme consisting of six stages is the basis of the VDSI (vas deferens sequence index), calculated as the mean value of all imposex stages of a population. It has been shown that imposex intensities, measured as the VDSI in a range of affected prosobranch species, show a highly significant correlation with TBT concentrations in ambient seawater, as demonstrated for the dog whelk in Fig. 2. Consequently, the degree of coastal TBT pollution can be assessed with high precision by a determination of imposex intensities in prosobranch populations. In addition, the calculation of the VDSI offers the opportunity to perform interspecific comparisons of TBT sensitivities of different species and to measure the reproductive capability of a given population (Oehlmann *et al.*, 1996b).

The periwinkle *Littorina littorea* develops a closely related virilization phenomenon as a response to TBT exposure, termed intersex (Bauer *et al.*, 1995). Intersex females are either characterized by male features on female pallial organs, specifically by an inhibition of the ontogenetic closure of the pallial oviduct or female sex organs are supplanted by the corresponding

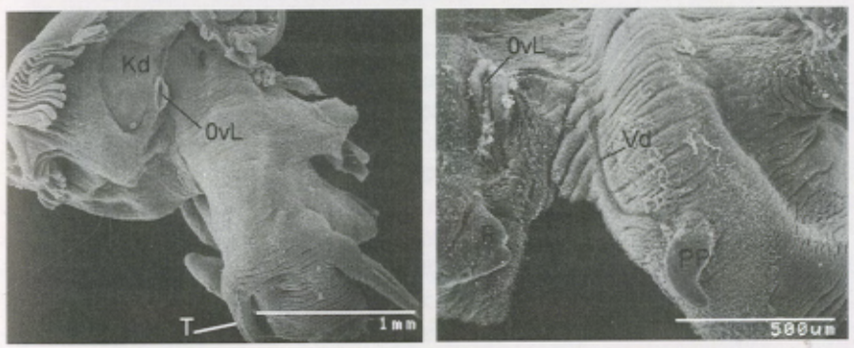

FIG. 1 *Hydrobia ulvae*. Scanning electron micrographs of females with their mantle cavity opened. Left: normal female without imposex; right: sterilized female in the final stage of imposex with blocked oviduct. Kd, capsule gland; OvL, ooparous opening of oviduct (open left; blocked right); PP, penis; T, tentacle; Vd, vas deferens.

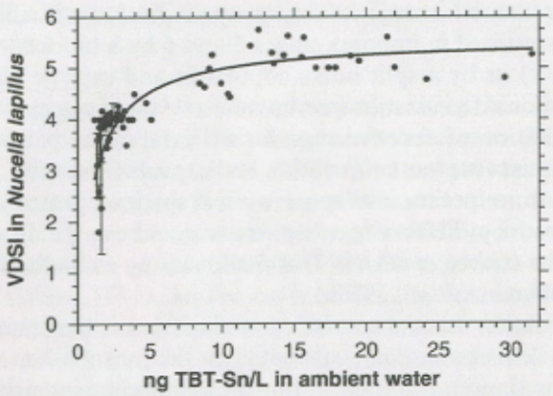

FIG. 2 *Nucella lapillus*. Relationship between aqueous TBT concentrations and imposex intensities: $y = (5.54x)/(1.12 + x)$; $n = 151$ population samples from 81 stations; $r = 0.688$; $p < 0.0005$.

male formations particularly by a prostate gland. Comparable to imposex, the intersex response is a gradual transformation of the female pallial tract, which can be described by an evolutionary scheme with four stages (Bauer *et al.*, 1995). Intersex development causes restrictions of the reproductive capability of females. In stage 1, a loss of sperm during copulation is possible and consequently reproductive success is reduced. Females in stages 2–4 are 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 produced by the species, as long as aqueous TBT levels are not beyond mortality threshold concentrations for the larvae (Matthiessen *et al.*, 1995).

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. A value of 0.0 indicates only normal females (stage 0) occur and no restrictions of the reproductive capability are to be expected. ISI values above 0 show that intersex-affected females can be found and that reproductive success may be reduced. ISI values are significantly correlated to ambient TBT concentrations and can therefore be used in parallel or as an alternative to imposex assessments for the determination of the degree of coastal TBT pollution, especially in regions with a comparatively high level of contamination where sensitive species such as dog whelks may already have become extinct. In these areas periwinkles are very common and can be sampled in sufficient numbers because *L. littorea* is more tolerant regarding high TBT levels. Imposex in dog whelks

and intersex in periwinkles have been used as combined biological markers for the convention-wide TBT effect monitoring of OSPAR (Oslo and Paris Commissions, 1996).

There is increasing evidence of TBT having also affected the community level, including negative effects even beyond the scale of populations. Waldock *et al.* (1999) 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 to a number of analyzed stations suggested a moderate improvement in environmental conditions over the sampling period, which was coincident with a marked decline in aqueous TBT concentrations at the same stations. However, reference to historical data indicated certain taxa that were previously frequent or common, especially snails, were only rarely recorded or still absent in the 1992 survey.

Far less attention has been paid to endocrine effects of TBT in freshwater ecosystems. The ramshorn snail, *Marisa cornuarietis*, and the freshwater mud snail, *Potamopyrgus antipodarum*, exhibit endocrine-mediated effects of TBT (Schulte-Oehlmann *et al.*, 1995). At the molecular level, TBT interferes with hormone metabolism, most probably by an inhibition of the cytochrome *P*-450-dependent aromatase, increasing the levels of androgens (Bettin *et al.*, 1996; Matthiessen and Gibbs, 1998; Morcillo *et al.*, 1999), while results from the early 1980s show that TBT inhibits the release of a neuroendocrine factor from the pleural ganglia, which is responsible for the suppression of penis formation in females, resulting in imposex development (Féral and Le Gall, 1982). Although the factor has not been identified, Oberdörster and Cheek (2001) were able to induce imposex in *Ilyanassa obsoleta* by administration of the neurohormone APGWamide. A possible explanation for these conflicting results may be 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 (Oehlmann and Schulte-Oehlmann, 2003).

It has been accepted that imposex is induced almost exclusively by TBT under field conditions (deFur *et al.*, 1999), although at least in the marine rock shell *Thais clavigera* (Horiguchi *et al.*, 1997) and the freshwater ramshorn snail *Marisa cornuarietis* (Schulte-Oehlmann *et al.*, 2000) not only TBT but also triphenyltin compounds (TPT) can promote imposex, while in other prosobranch species, such as *Nucella lapillus* and *Nassarius reticulatus*, TPT does not induce this virilization phenomenon (Schulte-Oehlmann *et al.*, 2000).

One of the most important lessons to be learned from the "TBT story" and its effects in mollusks is that EDCs may impact different levels of biological integrations from molecules to communities affecting also the survival of

populations in the field. Furthermore, the case history of TBT provides evidence for vertebrate-type steroids playing an important functional role in a number of invertebrate groups, including prosobranchs.

Since the EDIETA workshop, a number of further reports on the effects of organotin compounds in various taxonomic groups have been published, but still with a clear focus on mollusks within the invertebrates.

Several field studies were performed investigating the induction of imposex in the neogastropod genus *Thais*. Evans *et al.* (2001) recorded imposex in *Thais* spp. from each of the sites surveyed in Hong Kong and Singapore, and the majority of sampled stations in Phuket and Penang. The most advanced symptoms were those from shores close to commercial harbors, marinas, and mariculture sites, suggesting that TBT is a primary cause of the condition. All females were categorized as sterile from four sites in Hong Kong. There was evidence of decreasing symptoms of imposex with increasing distance from centers of shipping activity. Particularly, *T. bitubercularis* and *T. distinguenda* are recommended as indicators of TBT in Southeast Asia because of their sensitivity and wide distribution in the region. Bech (2002a) reported an enhancement of imposex in *T. distinguenda* from 1996 to 2000 in Phangna Bay, Thailand. The percentage of females with imposex increased in the populations over the investigation period, extending from 3.5 km in 1996 to 10 km from the harbor in 1999 and 2000. Bech *et al.* (2002) performed a translocation experiment with *T. distinguenda* taken from a pristine island in the Andaman Sea and tagged prior to translocation to an area of intense shipping activities. After 3 months 17% of the small size (less than or equal to 25 mm) and none of the big size class (>25 mm) had developed imposex. After 5 months the incidence of imposex in the big and small size classes increased steadily with time reaching 86 and 80%, respectively, after 1 year. To evaluate the range of effects in areas of increasing yachting activities, *T. distinguenda* was used as an indicator of imposex at two popular mooring sites for yachts at Phi Phi and Raja Island in Phangna Bay, Thailand (Bech, 2002b). The incidence of imposex was 100% at stations close to the mooring sites. A significant correlation was found between the distance from the sites and the incidence of imposex. Next to *T. distinguenda* imposex was also observed in further *Thais* species. Bech (2002a) described for *T. bitubercularis* an increasing intensity of imposex between 1996 and 2000. Ramasamy and Murugan (2002) reported imposex in *T. biserialis* from inside the Tuticorin port, India. The sex ratio was clearly biased with males being dominant. The frequency of imposex in females was 35%. The VDSI indicated the occurrence of initial imposex stages 1 and 2. In another survey along the New South Wales coast of Australia, Gibson and Wilson (2003) found imposex in *T. orbita* despite the fact that TBT was partially banned from antifouling paints for 10 years. As demonstrated by these authors, imposex was induced in this species over a period of 9 weeks in Sydney Harbor. *T. clavigera*,

sampled in Taiwan oyster mariculture areas, was also affected by imposex (Hung *et al.*, 2001). Imposex in *Thais bufo*, *T. rudolphi*, *T. tissoti*, and *Ocenebra bombayana* was investigated from the Saurashtra coast near Gujarat (Tewari *et al.*, 2002). The maximum percentage of imposex was observed in *T. rudolphi* with 47%.

In addition to the genus *Thais*, imposex was also observed in other marine neogastropods, particularly in snail species of the families Buccinidae, Muricidae, and Nassaridae. Gooding *et al.* (2003) recorded imposex in the mud snail *Ilyanassa obsoleta*. The incidence of imposex increased significantly when the snails were exposed to environmentally relevant concentrations of TBT (≥ 1.0 ng Sn/liter). Neogastropods like *Buccinum* sp. taken from harbors also exhibited imposex development (Strand and Asmund, 2003). A field survey on the development of imposex in the common whelk (*Buccinum undatum*) and the red whelk (*Neptunea antiqua*) was performed by Ten Hallers-Tjabbes *et al.* (2003). For the common whelk the authors found in the Southern Bight and the German Bight of the North Sea (at these locations sediment levels of organotins were also higher compared to other sites) the highest body levels of all organotin compounds and the highest imposex indices. In the red whelk, imposex development increased with shipping density, too. Chiavarini *et al.* (2003) sampled *Hexaplex trunculus* from the Sicilian coasts and observed a logarithmic correlation between TBT and imposex stages. Rilov *et al.* (2000, 2001) found very high imposex indices (VDSI 4–5) in the whelks *H. trunculus* and *Stramonita haemastoma* (= *Thais haemastoma*) taken from marinas of the Mediterranean coastline in Israel. A further field observation on imposex in *H. trunculus* was performed by Axiak *et al.* (2003). The authors observed the incidence of imposex in *H. trunculus* is related to body burdens and to the environmental levels of organotin as measured analytically.

Imposex in the netted whelk *Nassarius reticulatus* proved to be a useful bioindicator of TBT in polluted areas. The presence of functionally sterile females at highly polluted sites suggests that TBT-induced imposex may sterilize female *N. reticulatus*. Barroso *et al.* (2002) obtained samples of *N. reticulatus* from the west Portuguese coast. Imposex increased with the proximity of harbors. The percentage of imposex-affected females varied between 0 and 100%. The VDSI ranged from 0 to 5. Sterile females were found at 20% of the sampling stations. TBT and triphenyltin (TPT) female body burden varied from <20 to 1370 and from <10 to 256 ng Sn/g dry weight, respectively. Highly significant correlations were established between the TBT female residue and the VDSI. Barreiro *et al.* (2001) measured levels of imposex in *N. reticulatus* at 15 localities in northwest Spain. The masculinization of the gonadial section of the oviduct was graded in various stages (i.e., degree of convolution resembling the male seminal vesicle). This anomaly affected a substantial proportion of females (26.5%).

Both *Nassarius reticulatus* and *Potamopyrgus antipodarum* were used to monitor androgenic activities in sediments of the River Elbe (Schulte-Oehlmann *et al.*, 2001b). 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 4 weeks. Although TBT was the main responsible pollutant for imposex development, an even stronger biological effect than expected on the basis of analytically measured TBT concentrations was assessed for a number of sediments. This indicates a contribution of further, not yet identified compounds with androgenic activity to the observed imposex intensities in these freshwater sediments.

TBT also induced imposex in the dog whelk *Nucella lapillus* in a number of studies, confirming older reports (Barroso and Moreira, 2002; Birchenough *et al.*, 2002b; Santos *et al.*, 2002). Følsvik *et al.* (1999) investigated the occurrence of imposex in this species along the Norwegian coast between 1993 and 1995. The concentration of TBT in the gastropods from unaffected populations was below the detection limit (7 ng Sn/g dw). The concentration of TBT in dog whelks from affected populations was in the range of 48–1096 ng Sn/g dw. A positive relation between the concentration of TBT in dog whelks and the degree of imposex was found. *N. lapillus* became locally extinct on some shores adjacent to areas of high shipping/boating activity during the period of high TBT contamination in the 1980s and early 1990s (Birchenough *et al.*, 2002a). However, the species has now recolonized sites at which extinction occurred on the Isle of Cumbrae, the northeast coast of England, the Shetland Isles, and southwest England. There have also been substantial declines in the severity of imposex on adjacent shores where the species has persisted during this period. Moreover, imposex was described for the mangrove-dwelling muricid *Chicoreus capucinus* (Bech, 2002b) and in *Morula musiva*, *M. granulata*, *M. margariticola*, and *Thais rufotincta* (Bech, 2002a).

As known from several studies (Horiguchi *et al.*, 2004; Ide *et al.*, 1997; Strand *et al.*, 2003; Sudaryanto *et al.*, 2002) TBT accumulates in mollusks effectively. For example, the marine bivalve *Mytilus edulis* from the Gulf of Gdansk and from Nuuk harbor (West Greenland) showed high tissue residues with 68 ng Sn/g ww and 104 ng Sn/g ww, respectively (Albalat *et al.*, 2002; Strand and Asmund, 2003). Likewise, in the tissue of the mussel *Mytilus galloprovincialis* from the Adriatic Sea, Nemanic *et al.* (2002) measured TBT concentration up to 8.540 ng/g dw. TBT caused effects in *M. edulis* and *M. galloprovincialis* and in other the marine bivalves (e.g., *Mya arenaria*, *Ruditapes decussatus*). St. Jean *et al.* (2002a) used hemocyte functions to determine the effect of low concentrations (1, 3, and 6 ng Sn/L) of TBT and DBT on the immune system of adult *M. edulis* under

flowthrough conditions over 11 days. Their results support the hypothesis that both TBT and DBT can cause modulations of the internal defense system of mussels at concentrations as low as 1 ng Sn/liter after only a few days of continuous exposure. In a further study St. Jean *et al.* (2002b) established strong and sustained responses of hemocyte functions of blue mussels exposed to environmentally relevant, waterborne concentrations of TBT and DBT (0.04 to 0.67 n*M*), which is interpreted as an increase of their vulnerability to other environmental stressors and pathogens, even at low seawater temperatures (<5 °C), often present in high-latitude coastal waters. In addition to effects on the internal defense system, Jha *et al.* (2000a) found genotoxic effects of TBT to embryo–larval stages of *M. edulis*. Moreover, Jha *et al.* (2000b) suggested TBT was able to induce cytogenetic damage (sister chromatid exchanges and chromosomal aberrations) in this target species. The authors emphasized the need to evaluate the genotoxic potential of other endocrine-disrupting agents in different taxonomic groups.

In addition to *Mytilus* spp., organotin compounds affect other bivalve species. For example, the sex ratio in soft-shell clams (*Mya arenaria*) sampled from an intertidal harbor zone in the Saint Lawrence estuary (Canada) was significantly skewed toward males (Gagne *et al.*, 2003). Moreover, the condition and gonad-somatic indices, vitellin-like proteins in female gonads, and the capacity of female gonads to produce 17β-estradiol were significantly reduced at the harbor site with respect to the reference sites. The maturation status of male gonads was clearly delayed at the harbor site. Additionally, gonad tissue contained TBT at an average level of 109 ± 18 ng Sn/g dw at the harbor site while organotins were not detected from the reference sites. In laboratory experiments Coelho *et al.* (2001) studied the effects of sublethal TBT concentrations on the growth and development of the clam *Ruditapes decussatus* larvae. Therefore, larvae were exposed to a range of TBT nominal concentrations from 25 to 100 ng Sn/liter for a period up to 13 days. Growth and development were severely affected by TBT concentrations ≥25 ng Sn/liter. A 3- to 6-fold reduction of growth was observed in these early larval stages. A field study of Coelho *et al.* (2002) revealed TBT burdens in *R. decussatus*, sampled from the most important fishing harbor of the southern coast of Portugal, having deleterious developmental effects.

Moreover, in literature, TBT effects on the abalones *Haliotis madaka* and *Haliotis gigantean* were recorded. In this context Horiguchi *et al.* (2000) performed a field study with *H. madaka* evaluating possible endocrine disruption and considering the causal factors for the decline of abalone stocks in Japan. Abalone specimens were collected from a reference site and a site representative of declining abalone populations. Approximately 20% of the abalone from the polluted site were masculinized females with an ovo-testis. The masculinization of female abalone was similar to the imposex condition, typically induced in other gastropod mollusks by TBT and TPT from

antifouling paints. *In situ* exposure of abalone from the reference site caged near a dockyard in the polluted site for 7 months (from the immature to the mature stage) resulted in spermatogenesis in the ovary of approximately 90% of females.

Not only TBT but also TPT can promote imposex in the freshwater ramshorn snail *Marisa cornuarietis*, while in other prosobranchs, such as *Nucella lapillus* and *Nassarius reticulatus*, TPT does not induce imposex (Schulte-Oehlmann *et al.*, 2000). In addition to imposex development in marine and freshwater gastropods TBT and TPT induced a sharp decline in the number of embryos of the freshwater mudsnail *Potamopyrgus antipodarum* sheltered in its brood pouch in a time- and concentration-dependent manner in comparison to the control sediment (Duft *et al.*, 2003a). The organisms were exposed to artificial sediments, spiked with seven concentrations, ranging from 10 to 500 µg nominal TPT-Sn/kg dw and TBT-Sn/kg dw, respectively. The number of new, still unshelled embryos turned out to be the most sensitive parameter. For most parameters an LOEC of 10 µg/kg of each test compound was calculated. The EC_{10} resulted in even lower values for both substances. After 8 weeks for unshelled embryos an EC_{10} value of 0.03 µg TPT-Sn/kg was found. *P. antipodarum* seems to be highly sensitive to both endocrine disruptors TPT and TBT at environmentally relevant concentrations. Horiguchi *et al.* (2002) confirmed the induction of masculinization of the abalone *H. gigantea* within 2-month flowthrough exposure experiments with TBT and TPT in the laboratory. According to these findings, nominal concentrations of 100 ng TBT/liter and 100 ng TPT/liter caused significant spermatogenesis in ovaries of exposed females. There were also significantly more contracted primary oocytes observed in females exposed to either TBT or TPT than controls. The incidence of two types of unknown cells was also significant in females exposed to TPT. No significant histological changes were observed in testis of exposed males. This ovarian spermatogenesis caused by TBT and/or TPT resembles gastropod imposex and intersex. Remarkably high concentrations of TBT and TPT were observed in the head (including ganglia of the central nervous system), compared to concentrations in muscles. Accumulation of TBT and TPT in the head may disturb reproductive hormonal regulators through neuropeptides released from ganglia. The authors proposed that this fact, as well as possible aromatase inhibition, was one of the inducers for spermatogenesis in the abalone ovaries. Based on considerations of the steroid hormone theory of reproduction and in view of the disruption of endocrine systems in mollusks by organotins, Takeda (2000) investigated the effects of tributyltin on female apple snails (*Pomacea canaliculata*). Exposure to tributyltin resulted in imposex, and both a penis and a penis sheath were newly generated from the so-called vestigial penis. The same phenomenon was also induced by testosterone.

B. Insects: Insect Growth Regulators (IGRs) as Purposely Synthesized EDCs

The IGRs represent a group of insecticides, often referred to as third-generation insecticides, which were developed to intentionally mimic, block, or otherwise interact with the hormonal system of insects. They represent—in addition to selected organotin compounds—a second group of xenobiotics rated by the EDIETA workshop as a confirmed case for ED. The workshop report (deFur *et al.*, 1999) summarizes up to 47 references, mainly laboratory studies but also a number of field investigations with a focus on 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 runoff during or after agricultural application received little attention.

The IGRs consist of different classes of chemicals with specific modes of action, particularly of agonists and antagonists at the ecdysteroid receptor and of juvenile hormone (JH) analogs. Tebufenozide, methoxyfenozide, RH 5849, and other ecdysteroid receptor agonists induce symptoms of hyperecdysonism in terrestrial insects, delaying postembryonic development and nymphal–adult intermediates. Paradoxically, even though most insects and other arthropods use ecdysone as a molting hormone, tebufenozide is selectively toxic to the Lepidoptera (Dhadialla *et al.*, 1998). Ecdysteroid antagonists with the examples of azadirachtin or KK-42 prevent normal diapause induction and induce an early termination of diapause or a precocious metamorphosis, while 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.

Even though IGRs deploy these marked effects in insects, there are major terrestrial invertebrate classes, however, for which there are no apparent data on the impact of these compounds, e.g., earthworms and mollusks. There are also no reported incidents of effects of nonpesticidal endocrine disruptors, which are present in the environment on terrestrial invertebrates. Occasionally, chitin synthesis inhibitors (CSI), such as diflubenzuron, other benzylphenyl ureas, and related compounds (Ishaaya, 1992), have been misreported as EDCs. Although they interfere with molting as an endocrine-regulated process in arthropods, the mechanism of CSI action is purely nonendocrine. CSI inhibit one of the steps of chitin synthesis selectively. Because this synthesis usually takes place at or during the time of molting, CSI cause death during the molt, resembling the same effect as endocrine mimicking IGRs, albeit not via an endocrine pathway.

Dinan *et al.* (2001) developed the B-II bioassay as a screening tool for detecting potential insect growth regulators acting as ecdysteroid receptor agonists and antagonists. Based on an ecdysteroid-responsive cell line from *Drosophila melanogaster* about 80 potential environmental contaminants,

including industrial chemicals, pesticides, pharmaceuticals, phytoestrogens, and vertebrate steroids, are compared with data for known (ant)agonists. Apart from androst-4-ene-3,17-dione, vertebrate steroids were inactive at concentrations up to 10^{-3} *M*. The vast majority of xenobiotics did not possess an (ant)agonistic activity. Among the industrial chemicals, antagonistic activity was observed for bisphenol A (BPA) (EC_{50} of 1.0×10^{-4} *M*) and diethylphthalate (EC_{50} of 2.0×10^{-3} *M*). Some organochlorine compounds were also characterized by a weak antagonistic activity, including DDE, dieldrin, and lindane (EC_{50} of 3.0×10^{-5} *M*). The only pharmaceutical showing any detectable antagonist activity was 17α-ethinylestradiol (EE2). In the context of recent publications on potential endocrine disruption in marine and freshwater arthropods, these findings suggest that for some compounds (e.g., diethylstilbestrol), ecdysteroid receptor-mediated responses are unlikely to be involved in producing chronic effects.

Hahn *et al.* (2001) performed laboratory experiments with the nonbiting midge *Chironomus riparius* to determine the effects of the molting-hormone agonistic insecticide tebufenozide (1–100 µg/liter). After static contamination of first-instar larvae the NOEC, LOEC, and LC_{50} values were 13.2, 17.4, and 21.1 µg/liter, respectively. Semistatic exposure of fourth-instar larvae revealed a lower susceptibility of elder larvae (NOEC 30 µg/liter, LOEC 60 µg/liter, and LC_{50} 81.9 µg/liter). Pupal mortality in the semistatic exposure scheme was twice as high in males as in females during a 100 µg/liter treatment. This sex-related difference probably resulted from the endocrine activity of tebufenozide.

Moreover, studies were performed with larvae in the silkworm *Bombyx mori* (Lepidoptera) (Obara *et al.*, 2002). For example, Dedos *et al.* (2002a,b) studied the relationship between fenoxycarb levels and general esterase (GE) activity, juvenile hormone esterase (JHE) activity, and induction of permanent fifth instar (dauer) larvae. Fenoxycarb (1 µg per animal) was applied to fifth instar of *B. mori*. This fenoxycarb treatment induces dauer larvae with persistently increased hemolymph GE and JHE activities for the rest of the fifth instar.

IV. Further Cases of Endocrine Disruption in Aquatic and Terrestrial Invertebrates

Next to the effects of TBT and xenoestrogens in prosobranchs and of IGRs in insects, 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 (deFur *et al.*, 1999) summarizes up to 56 studies in which

ED may have occurred, although nonendocrine mechanisms are also possible for the observed effects.

The crustaceans represent the systematic group providing the majority of cases suspect for ED. Whereas the examples for the aquatic environment are almost balanced between freshwater and marine species, only two studies report comparable effects in terrestrial arthropods.

Since the publication of the EDIETA report, numerous new cases for ED in invertebrates have been documented in the literature prior to August 2003. These are described in more detail but without the intention of providing a complete list, because still unpublished data from the currently ongoing and still increasing research efforts for the identification of new suitable test species and sensitive end points could not be considered.

A. Porifera

Within the scope of a laboratory study Hill *et al.* (2002) examined toxic effects of ethylbenzene, nonylphenol, and BPA, which are known to be hormonally active in many animal species, on growth and development of the freshwater sponge *Heteromyenia* sp. The authors found a common developmental abnormality when sponges were treated with each of these compounds. The three compounds also caused significant reductions in growth rates. Germination of *Heteromyenia* sp. was completely inhibited in the 3 mg/liter ethylbenzene treatment, and when exposed to 80 mg/liter BPA. Lower concentrations resulted in malformed water vascular systems in several replicates.

B. Rotatoria

Preston *et al.* (2000) developed a 96-hr reproductive assay using the freshwater rotifer *Brachionus calyciflorus*. Cadmium, chlorpyrifos, naphthol, pentachlorophenol, estradiol, methoprene, precocene, nonylphenol, flutamide, and testosterone were examined for effects on asexual and sexual reproduction. Flutamide, testosterone, and nonylphenol inhibited fertilization of sexual females at concentrations of 1, 10, and 50 µg/liter, respectively. Sexual reproduction was inhibited with no effects on asexual reproduction, increasing the likelihood that these specific reproductive effects occurred through an endocrine mechanism. In a further study using *B. calyciflorus* Radix *et al.* (2002) found a decrease in the total number of females after exposure to ethinylestradiol (489 µg/liter), nonylphenol (130 µg/liter), and testosterone (2560 µg/liter).

C. Annelida

As shown in Table II, in the EDIETA report (deFur *et al.*, 1999) only one paper is presented in which endocrine disruption in annelids was studied. Only a few papers dealing with ED in annelids can be found in the literature. In most cases marine organisms were considered. Depledge and Billinghurst (1999) reported effects (increase in egg production and reduction of egg availability) of the xenoestrogen 4-nonylphenol on the polychaete worm *Dinophilus gyrociliatus*. Jha *et al.* (2000a) exposed embryo–larval stages of the polychaete *Platynereis dumerilii* to a range of concentrations of TBT, and analyzed the organisms for cytotoxic (proliferation rate index) and genotoxic (sister chromatid exchanges and chromosomal aberrations) effects. According to these findings TBT is toxic and genotoxic to embryo and larval stages. *P. dumerilii* is more sensitive compared to the target species *M. edulis*. Hagger *et al.* (2002) suggested for *P. dumerilii* dose-dependent effects for genotoxic and cytotoxic end points in relation to TBT exposure. Meador and Rice (2001) exposed juveniles of the opheliid polychaete, *Armandia brevis*, to sediment-associated TBT for 42 days. They determined an LOEC for growth of 101 ng/g dw; the sediment concentration causing a 25% inhibition in growth was 93 ng/g dw. A dose–response relationship was also determined for polychaete net weight and TBT in tissue. The tissue residue associated with a 25% reduction in growth was 2830 ng/g dw. When comparing these results with previous reports the authors noticed that juveniles are approximately three times more sensitive than adults to TBT exposure.

In addition to the marine annelids, terrestrial earthworms (*Dendrobaena octaedra* and *Eisenia fetida*) were used to assess effects of ED on this phylum, however, only two studies were found. Ricketts *et al.* (2003) performed research on the identification and purification of vitellogenin in *E. fetida*, which will allow the monitoring of any effects on the reproductive capacity of earthworm species resulting from exposure to environmental estrogens. Widarto *et al.* (2003) observed a significant effect of nonylphenol (50 mg/kg dry soil) on the population growth rate of the lumbricid earthworm *D. octaedra* when a simple two-stage model was used.

TABLE II
Laboratory Study in Which Endocrine Disruption May Be Occurring in Annelids[a]

Species	Contaminant	Concentration (range), effects	Lab/field	Reference
Platynereis dumerilii	Volatile organic compound from crude oil	0.3 mg/liter per compound; induction of characteristic spawning behavior	Lab	Beckmann *et al.*, 1995
Nereis sucinea				

[a]Modified from deFur *et al.* (1999).

D. Mollusca

In the EDIETA report (deFur *et al.*, 1999) only two publications concerning endocrine disruption in mollusks (*Lymnaea stagnalis* and *Mytilus edulis*) were found, compared to the numerous studies on organotin effects (cf. Section III.A) (Table III).

Since the EDIETA workshop in late 1998, numerous publications on endocrine disruption in mollusks have been carried out. Most of them used gastropods (particularly prosobranchs) to investigate the effects of ED (mainly alkylphenols and bisphenol A). Oehlmann *et al.* (2000) exposed the freshwater prosobranch *Marisa cornuarietis* to BPA and octylphenol (OP) at nominal concentrations between 1 and 100 µg/liter in experiments with sexually mature snails over 5 months and also within a complete life cycle for 12 months. The xenoestrogens induced a complex syndrome of alterations in female *Marisa* referred to as "superfemales" at the lowest concentrations. Affected specimens were characterized by the formation of additional female organs, an enlargement of the accessory pallial sex glands, gross malformations of the pallial oviduct section (Fig. 3) resulting in an increased female mortality, and a massive stimulation of oocyte and spawning mass production. An exposure to OP resulted in inverted U-type concentration response relationships for egg and spawning mass production.

In addition to *Marisa*, Oehlmann *et al.* (2000) exposed adults of the marine dog whelk *Nucella lapillus* to BPA and OP for 3 months with nominal concentration in the range of 1–100 µg/liter. As in *Marisa*, superfemales with enlarged accessory pallial sex glands and an enhancement of oocyte

TABLE III
Representative Laboratory Studies in Which Endocrine Disruption May Be Occurring in Mollusks[a]

Species	Contaminant	Concentration (range), effects	Lab/field	Reference
Lymnaea stagnalis (adult)	DDT, MCPA	50, 500 µg/liter (DDT); 10, 100 mg/liter (MCPA); fecundity alterations	Lab	Woin and Bronmark, 1992
Mytilus edulis (adult)	Cd	100 µg/liter; spawning stimulation, inhibition of gonadal development	Lab	Kluytmans *et al.*, 1988

[a]Modified from deFur *et al.* (1999).

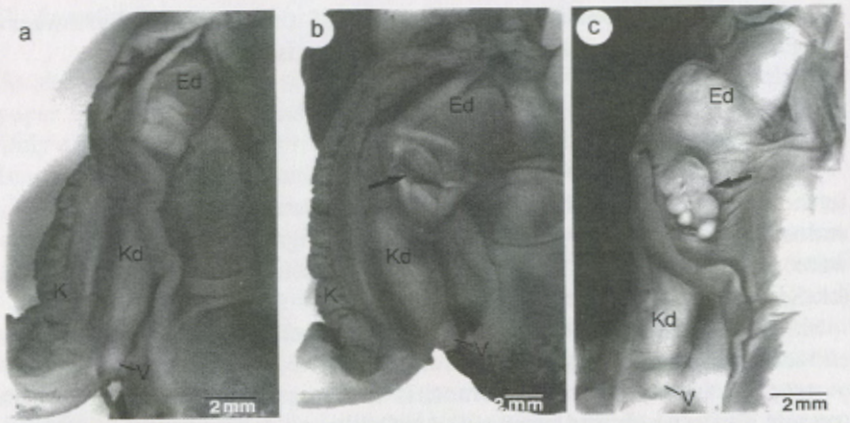

FIG. 3 *Marisa cornuarietis*. Photographs of a control female (a) and of BPA-treated "superfemales" (b and c) with opened mantle cavities. In (b) and (c) a rupture in the wall of the pallial oviduct occurs (arrow) with an additional protrusion of the spawning mass in (c). Ed, albumen gland; K, gill; Kd, capsule gland; V, vagina.

production were observed. No oviduct malformations were found, probably due to species differences in the gross anatomical structure of the pallial oviduct. A lower percentage of exposed specimens had ripe sperm stored in their vesicula seminalis and additionally male *Nucella* exhibited a reduced length of penis and prostate gland when compared to the control. Because statistically significant effects were observed at the lowest nominal test concentrations (1 µg BPA or OP/liter), it can be assumed that even lower concentrations may have a negative impact on the snails. This assumption was confirmed by Schulte-Oehlmann *et al.* (2001a) reporting NOEC and LOEC values for the induction of superfemales in *Marisa* of 7.9 and 48.3 ng/liter, respectively.

The superfemale response was confirmed for the freshwater mudsnail *Potamopyrgus antipodarum* in laboratory experiments with the xenoestrogens BPA, OP, and 4-*n*-nonylphenol (NP) following exposure via sediments (Duft *et al.*, 2003b). The test species exhibited a distinct increase in the number of embryos sheltered in its brood pouch in a time- and concentration-dependent manner in comparison to the solvent control sediment for BPA and OP. The number of early, still unshelled embryos turned out to be the most sensitive parameter. The observed LOEC was equivalent to the lowest administered concentration (1 µg/kg for each test compound) after 8 weeks of exposure. The EC_{10} values for unshelled embryos after 10 weeks were 0.03 µg BPA/kg and 0.2 ng OP/kg. For NP, there was no clear concentration-dependent response. The LOEC in the experiments with NP was 10 µg/kg.

Tillmann *et al.* (2001) found a suppression of imposex development in *Marisa*, *Nucella*, and *Nassarius reticulatus* if snails were coexposed to TBT and either to the antiandrogen cyproterone acetate (CPA) (1.25 mg/liter) or vinclozolin (VZ) (0.03–1.0 µg/liter). If administered alone, the antiandrogens induced a number of biological responses in all three species. The length of the penis and of accessory male sex organs (e.g., penis sheath, prostate) was significantly reduced. For *Marisa*, this effect occurred only in sexually immature specimens and was reversible as the males attained puberty. Typical androgen-mediated responses (imposex development, delayed spermatogenesis, tubulus necrosis of the testis with orchitis, and Leydig cell hyperplasia) were partially or totally suppressed by a simultaneous administration of CPA. In the two marine species even adult, sexually mature males responded to antiandrogens with a reduction of the male sex organs and an advancement of the sexual repose phase. The results for CPA and VZ are compared with the effects of an exposure to xenoestrogens (BPA, OP) and xenoandrogens (TPT, TBT) in the same species. Each group of endocrine disruptors induces a characteristic set of toxicological effects in prosobranch snails, which can be used as end points in an organismic invertebrate test for the identification of endocrine mimetic test compounds. Estrogens cause primarily an induction of superfemales resulting in increased female mortality by the enhancement of spawning mass and egg production. The main effects of androgens are a virilization of females by imposex development and a marked decrease of fecundity. Compared with estrogens and androgens, the antiandrogen responses seem to be less drastic and might have—in contrast to the two other disruptor classes—no biologically significant effects at the population level.

In a field study Morcillo and Porte (1999) determined testosterone/estradiol tissue levels and cytochrome *P*-450-dependent aromatase activity in the marine gastropod *Bolinus brandaris*, collected from the Mediterranean coast (Spain), with different degrees of imposex development. A strong reduction of estradiol levels was seen in both males and females from the organotin-polluted site in comparison to those of the reference site, whereas weaker differences between the sites in testosterone levels were revealed. According to these findings (alterations in sex hormone levels, but no changes in aromatase activity were recorded) the authors indicate that mechanisms other than inhibition of aromatase activity might be involved in the development of imposex in *B. brandaris*.

Next to VZ the pesticide Paraquat (PQ) turned out to be endocrinically active in snails. Bacchetta *et al.* (2002) determined the influence of PQ on the ovipository activity of the freshwater snail *Physa fontinalis* and on histological effects of these snails. Specimens exposed to PQ (0.125–0.5 mg/liter) continued to be reproductively active, but the number of egg masses and eggs laid decreased significantly. Mortality was almost the same in all the

experimental lots, but was significantly related to the production of egg masses only in the controls. The histological analysis showed a clear trend among PQ concentrations and degenerating oocytes, but no visible effects on the male sex line were observed.

Some studies report the effects of alkylphenols in bivalves. Nice *et al.* (2000) observed a delayed development to a particular larval stage (D-shape) of the Pacific oyster, *Crassostrea gigas*, when the bivalves were exposed to a range of concentrations of 4-nonylphenol (0.1–10,000 µg/liter). Moreover the compound caused a decrease in survival rate and malformed D-larvae of *C. gigas*. In a further laboratory study the exposure to 1 and 100 µg/liter nonylphenol at Days 7 to 8 postfertilization resulted in a change of the sex ratio toward females and in an increased incidence of hermaphroditism (Nice *et al.*, 2003).

On the biochemical level, effects of NP were used as a biomarker indicating estrogen disruption with bivalves serving as bioindicators. Blaise *et al.* (1999) demonstrated an induction of vitellogenin-like proteins in the hemolymph of the endobenthic soft-shell clam *Mya arenaria* by NP and other test chemicals (pentachlorophenol and 17β-estradiol). These findings were also approved in clams collected at specific intertidal stations of the Saguenay Fjord (located in Canada on the north coast of the Saint-Lawrence Estuary) during temporal and spatial surveys, which appear to indicate the presence of (anti)estrogenic chemicals in the Saguenay Fjord. Gauthier-Clerc *et al.* (2002) concluded from their field survey in the Saguenay Fjord that the persistent dysfunction of a vitellogenic process in *M. arenaria* was closely related to the exposure to antiestrogenic contaminants. In addition to *M. arenaria*, the zebra mussel *Dreissena polymorpha* is commonly used as a bioindicator of persistent organic pollutants in freshwater ecosystems in Europe and North America (Binelli and Provini, 2003). Mussels sampled in Pallanza Bay (close to a DDT-manufacturing plant), Lake Maggiore (Italy), exhibited marked oocyte degeneration suggesting that DDT has endocrine-disrupting effects in this species (Binelli *et al.*, 2001).

E. Crustacea

In the EDIETA report (deFur *et al.*, 1999) there is a large body of literature on the effects of certain chemicals (alkylphenols, heavy metals, phthalates, pesticides, and PCBs) on the crustacean endocrine system (Table IV).

Numerous studies using the cladoceran genus *Daphnia* can be found in the literature. Baldwin *et al.* (2001) exposed *Daphnia magna* in a life-cycle test for 21 days to the ecdysteroids, 20-hydroxyecdysone (20-E), the accepted molting hormone, and ponasterone A (PoA), an ecdysteroid found in some crustaceans and many plants. PoA elicited effects similar to those of 20-E at approximately 10 times lower concentrations. The effects of PoA on

TABLE IV
Representative Laboratory and Field Studies in Which Endocrine Disruption May Be Occurring in Crustaceans[a]

Species	Contaminant	Concentration (range), effects	Lab/field	Reference
Daphnia magna (multigeneration)	Diethylstilbestrol (DES)	0.031–0.5 mg/liter; reduced molting frequency, reduced fecundity	Lab	Baldwin *et al.*, 1995
Daphnia magna (adults)	4-Nonylphenol	25, 50, 100 µg/liter; reduced fecundity	Lab	Baldwin *et al.*, 1997
Daphnia magna (juveniles)	Cadmium	5, 10, 20 µg/liter; elevated ecdysteroid levels	Lab	Bodar *et al.*, 1990
Daphnia magna	Cadmium	0.5–50 µg/liter; delayed reproduction, reduced size of neonates, increased brood size	Lab	Bodar *et al.*, 1988
Daphnia magna	Phthalate esters	1 mg/liter; no effects on survival, growth, fecundity	Lab	Brown *et al.*, 1998
Rhithropanopeus harrisii, larval stages to adult	*S*-Methoprene (JH-analog)	100 µg/liter; mortality, increased intermolt duration	Lab	Celestial and McKenney, 1994
Daphnia magna (prenatal–adult)	Nonylphenol	0.018–0.32 mg/liter; mortality of adults and offspring, reduced brood size, reduction in length	Lab	Comber *et al.*, 1993
Daphnia pulex, *Eurytemora affinis* (neonate–adult)	Dieldrin	1–10 µg/liter; mortality, time to first reproduction, reduced fecundity, increased time to maturity, reduced number of broods	Lab	Daniels and Allan, 1981
Daphnia magna (juvenile–adult)	Endosulfan	0.12–0.31 mg/liter; reduced growth, decreased survival, reduction in brood size and number broods, delayed maturation	Lab	Fernandez-Casalderry *et al.*, 1993

(continued)

t	Concentration (range), effects	Lab/field	Reference
ıon	0.15–0.62 mg/liter; reduced rates of filtration and ingestion	Lab	Fernandez-Casalderry *et al.*, 1994
	–; abnormal coloration	Lab	Fingerman and Fingerman, 1978; Hanumante *et al.*, 1981; Staub and Fingerman, 1984
	4, 20 mg/liter; impairment of reproduction, reduction in molt frequency	Lab	Fitzmayer *et al.*, 1982
	Sublethal; supression of growth and reduced egg production	Lab	Johnston, 1987
	0.2 mg/liter; reduced fecundity, arrested development of juveniles	Lab	Kersting, 1975
	2–125 µg/liter; mortality, reduced growth, delayed brood release, reduced brood size	Lab	McKenney and Celestial, 1996
	0.1–1000 mg/liter; mortality, inhibition of larval development	Lab	McKenney and Matthews, 1990
	–; Hyperglycemia	Lab	Nagabhushanam and Kulkarni, 1981
ol	0.062–0.5 mg/liter; mortality, reduction in fecundity, reduced elimination of testosterone metabolites	Lab	Parks and LeBlanc, 1996
	0.015–0.04 mg/liter; retardation of regenerative limb growth and molting, increase in weight and calcium content of cast exuviae	Lab	Reddy *et al.*, 1991, 1992; Nagabhushanam *et al.*, 1990
	10 mg/liter; suppression of ovarian growth	Lab	Sarojini *et al.*, 1994
	1–5, 10, 20 mg/liter; reduced fecundity and growth	Lab	Schober and Lambert, 1977
	10–100 µg/liter; reduction in fecundity, developmental abnormalities	Lab	Shurin and Dodson, 1997
	0.5–500 µg/liter; retardation of regenerative limb regeneration and molting, abnormalities in the regenerates	Lab	Weis *et al.*, 1987
ry, lead	0.1, 1.0 mg/liter; retarded limb regeneration (Cd and Hg only)	Lab	Weis, 1976
	1, 3, 5 mg/liter; retarded limb regeneration	Lab	Weis, 1980
	0.05–0.20 mg/liter; increased duration of intermolt period	Lab	Zou and Fingerman, 1997a
henol	0.01–0.04 (4-octylphenol); 0.05–0.2 mg/liter (lindane); no effects on molting	Lab	Zou and Fingerman, 1997a
242,	0.05–0.2 (PCB29); 0.05–0.1 (Aroclor1242);	Lab	Zou and Fingerman, 1997b

daphnids mimicked those of 20-E except PoA reduced fecundity in the F_2 generation and 20-E had no effect. Both exogenous 20-E (260–1040 m*M*) and PoA (3.4–27 n*M*) show similar effects, including premature death associated with incomplete ecdysis, and the overall difference in toxicity is most likely due to receptor affinity. Using the antiandrogen cyproterone acetate (0.3–5 μ*M*) on *D. magna* (adults), a molt-independent growth reduction and reduced offspring numbers were reported (LeBlanc and McLachlan, 1999). Hence, chemicals capable of binding to the vertebrate androgen receptor would also elicit toxicity to crustaceans by binding to specific steroid hormone receptors in an antagonistic manner. Additional studies were performed to determine effects of (xeno)hormones on daphnids. As demonstrated by Olmstead and LeBlanc (2000), the development of secondary sex characteristics in daphnids can be altered by chemical exposure. The steroidal vertebrate androgen androstenedione (6.2–25 μ*M*) stimulates the development of the first antennae in males of *D. magna*. Exposure of female daphnids to the nonsteroidal vertebrate xenoestrogen diethylstilbestrol (0.75–3 μ*M*) and the insect juvenile hormone analog methoprene (0.08–0.32 μ*M*) stimulated development of the abdominal process. A further study was undertaken by Olmstead and LeBlanc (2002) to elucidate the hypothesis of the crustacean juvenoid hormone, methyl farnesoate, being a male sex determinant in *D. magna*. Continuous exposure to aqueous concentrations of methyl farnesoate (52–400 n*M*) stimulated a concentration-dependent production of male-containing broods of organisms. Olmstead and LeBlanc (2003) demonstrate that insecticidal juvenile hormone analogs (JHA) mimic the action of the crustacean juvenoid hormone methyl farnesoate, resulting in the inappropriate production of male offspring. When daphnids were exposed chronically (3 weeks) to sublethal concentrations of the JHA pyriproxyfen, the percentage of males produced by exposed maternal organisms increased. Furthermore, styrenes have the potential to impair crustacean populations in the aquatic environment (Tatarazako *et al.*, 2002). The authors found reduced offspring numbers in *Ceriodaphnia dubia* bred in polystyrene cups. Styrene dimers and trimers at concentrations of 0.04–1.7 μg/liter affected *C. dubia* fertility (25% reduction after 7 days).

Furthermore, amphipods (mainly *Gammarus* spp.) were used as test organisms (Gross-Sorokin *et al.*, 2003). Gross *et al.* (2001) performed a field survey to determine whether the sexual development of the freshwater crustacean *Gammarus pulex* was affected below sewage treatment works (STW) previously known to contain endocrine-disrupting chemicals in their effluent. Females collected from a site known to elicit high estrogenic responses in vertebrates displayed an abnormal structure of oocytes in vitellogenesis. Furthermore the body size was significantly shorter and the male/female size differential was significantly reduced below one of the STW. Watts *et al.* (2001b) determined the effects of the xenoestrogens EE2 and BPA on

the survival and reproductive behavior of the amphipod *G. pulex* in a series of bioassays. The reproductive behavior was disrupted only at comparatively high concentrations where it would be unrealistic to attribute the effects to an endocrine-mediated process. Consequently, changes in precopulatory guarding do not seem to be suitable for detecting the presence of xenoestrogens known to cause endocrine disruption in vertebrates in water.

Data on effects of endocrine disrupters in other crustaceans such as amphipods, copepods, cirripedes, and decapods are available as well. Brown *et al.* (1999) performed laboratory experiments with the marine amphipod *Corophium volutator* (juveniles – adults) and 4-nonylphenol (NP). At the lowest NP exposure of 10 μg/liter the density of surviving specimens was reduced and growth was retarded. Fertility of female *C. volutator* increased in NP-exposed populations. Sex ratio was not affected by NP exposure; however, the second antennae of exposed male animals were significantly longer than those of control animals. The authors concluded that large antennae will result in a restriction of movement in males, hence, those males are potentially at a selective disadvantage in the wild being more vulnerable to predation. A 21-day life-cycle test using the marine copepod *Tisbe battagliai* did not culminate in effects on survival, development, reproduction, and sex ratios when this species was exposed to environmentally relevant levels of steroidal estrogen agonists, or to related synthetic receptor agonists (Pounds *et al.*, 2002). Breitholtz and Bengtsson (2001) did not find any evidence of endocrine-disruptive activity in the copepod *Nitocra spinipes* exposed to the estrogens 17β-estradiol (E2) (0.0016–0.16 mg/liter) and EE2 (0.0005–0.05 mg/liter). The only significant effect detected at the highest diethylstilbestrol concentration (0.03 mg/liter) was a decreased number of females delivering nauplia after 15–18 days of exposure. Breitholtz and Wollenberger (2003) investigated effects of polybrominated diphenyl ethers (PBDEs) in the same species on development and reproduction. Copepods exposed for 6 days to PBDEs were characterized by a decreased larval development rate ($\geq$0.013 mg/liter BDE-47 and $\geq$0.03 mg/liter BDE-99). Within a full life-cycle test (less than or equal to 26 days exposure) the population growth rate in the copepods was reduced at 0.04 mg/liter BDE-47.

Moreover, cirripeds were used to assess the potential for endocrine disruption in aquatic environments. Billinghurst *et al.* (2000) examined the effects of exposure to 4-NP (0.01–1 μg/liter) and E2 (1 μg/liter) on the marine cirriped *Balanus amphitrite*. In particular, elevated levels of the cypris major protein (CMP), which is related to barnacle vitellin, were measured. The authors conclude that CMP and perhaps other vitellin-like proteins are potential biomarkers of low-level estrogen exposure in crustaceans. Another cirriped, *Elminius modestus*, was used in the laboratory to determine effects of endocrine disrupters on crustaceans (Billinghurst *et al.*, 2001). Exposure of larval stages to NP (0.01–10 μg/liter) and estradiol (10 μg/liter) resulted in altered

timing of larval development. Oberdörster *et al.* (2000) observed a significant increase of vitellin in female grass shrimps (*Palaemonetes pugio*) at 63 µg/liter of the PAH pyrene (exposure for 6 weeks). In addition to grass shrimp, several other crustacean species were screened and cross-reactivity was found, including blue crab (*Callinectes sapidus*), mud crab (*Rhithropanopeus harrisii*), red swamp crayfish (*Procambarus clarkii*), and *Daphnia magna*.

F. Insecta

Table V summarizes those publications on EDC effects in insects cited in the EDIETA report (deFur *et al.*, 1999). Since the EDIETA workshop research on ED effects in chironomids has increased interest. Many authors emphasized the suitability of the nonbiting midge *Chironomus riparius* as a model organism for investigating effects of endocrine-disrupting chemicals in aquatic insects. A laboratory study of Hahn and Schulz (2002) resulted in sex-specific effects of TBT (0.01–5 µg as Sn/liter) on molting-hormone biosynthesis and imaginal-disc development of *C. riparius*. Ecdysteroid synthesis decreased significantly in female larvae at all concentrations (50, 500, and 5000 ng TBT as Sn/liter), whereas a significant increase of biosynthesis rate occurred in male larvae in the 500 ng/liter treatment. *In vivo* experiments with development of the genital imaginal disc within a 48-hr exposure period revealed a significantly slower development in female larvae and a significantly faster development in male larvae at all concentrations tested (10, 50, 200, and 1000 ng TBT as Sn/liter). The LC_{50} (48 hr) was 25 µg/liter. The use of vitellogenesis as a marker for possible effects of endocrine-disrupting agents was tested with *C. riparius*. In this context Hahn *et al.* (2002) determined in a semistatic test system using BPA (1–3000 µg/liter) and NP (1.9–2000 µg/liter) an alteration of vitellogenin/vitellin production in males. Yolk concentrations in males were lowered by 20–25% after exposure to BPA at concentrations of 1, 100, and 3000 µg/liter. NP contamination caused an inverted dose–response curve. Female yolk protein contents were affected only in the highest BPA treatment, where yolk immunoreactivity was reduced by about 10% compared to control. In a two-generation experiment using the nonbiting midge *C. riparius* and EE2 and BPA, Watts *et al.* (2001a) were able to demonstrate that median emergence times (EmT_{50}) and the percentage of adult emergence were affected by EE2 and BPA exposure. These effects were primarily associated with the second generation of test animals, most notably in the BPA study, where the emergence of male and female adults was significantly delayed at concentrations ranging from 78 ng/liter to 750 µg/liter. At very low concentrations (1 ng/liter) of EE2, both the first and second generation of adults emerged significantly earlier than control animals. However, these end points, although validated as indicators of general

TABLE V
Representative Laboratory and Field Studies in Which Endocrine Disruption May Be Occurring in Insects[a]

Species	Contaminant	Concentration (range), effects	Lab/field	Reference
Chironomus tentans (all life stages)	4-Nonylphenol	12.5–200 µg/liter; reduced survival at high concentrations	Lab	Kahl *et al.*, 1997
Macromia cingulata (larvae)	Tannery and paper pulp mill effluent	5, 10, 15, 20, 20%; shortened time to first molt (tannery), arrested larval molting (paper pulp)	Lab	Subramanian and Varadaraj, 1993
Chironomus riparius (larvae–adult)	Phthalate esters	100, 1000, 10,000 mg/kg dw; no effects on survival, development, or emergence	Lab	Brown *et al.*, 1996
Chironomus spp.	Industrial effluent-containing metals	Ambient at site; higher level of mentum deformities in exposed larvae in both field and lab conditions	Field and Lab	Dickman and Rygiel, 1996
Chironomus thummi (larvae)	Organic and inorganic pollutants	Ambient at site; prevalence of morphological deformities related to pollutants	Field	de Bisthoven *et al.*, 1995
Chironomus spp.	Possible exposure to pollutants	Ambient at site; deformed mouth parts, heavily pigmented head capsules, unusually thick head capsule and body wall	Field	Hamilton and Seather, 1971

[a]Modified from deFur *et al.* (1999).

sediment toxicity, did not turn out to be well suited to detect the estrogenic effects of EE and BPA in *C. riparius*.

Many field studies provide evidence that mouthpart deformities in chironomid larvae are a sublethal response to pollution offering the opportunity to use this end point in programs for monitoring sediment quality. During laboratory studies, however, deformities were induced in only a few single pollutant exposures. These deformities develop at the endocrine-regulated molting stage and disruption of this complex process is likely at the base of their ontogeny. In laboratory bioassays Meregalli *et al.* (2001) investigated mouthpart deformities of *C. riparius* when larvae were exposed to NP (10–100 µg/liter). Mouthpart deformities result from a physiological disturbance during larval molting. Survival of the larvae was not affected by the tested concentrations, but the frequency of mentum deformities increased significantly. However, no adverse effects (survival and deformity induction) were found when *C. riparius* larvae were exposed to EE2 (1–100 µg/liter) (Meregalli and Ollevier, 2001). In a laboratory study Vermeulen *et al.* (2000) exposed instar II and III *C. riparius* larvae to sublethal concentrations of lead, mercury, and β-sitosterol. A significant deformation response was induced in the pecten with lead and mercury (50 µg Hg/liter and 100 mg Pb/liter, respectively). Both metals retarded molting. Exposure to sublethal concentrations of β-sitosterol did not result in any effect on deformation or molting.

In addition to publications on ED effects in chironomids, one paper was dedicated to Lepidoptera as the test organisms. Kirkbride-Smith *et al.* (2001) investigated the impact of the hormones estradiol, methyltestosterone, and thyroxin on growth, development, and reproduction of the tomato moth, *Lacunaria tolerance*. Administration of thyroxin and estradiol (dietary concentration of 1 mg/kg) caused an increase in the length and a reduction in the weight of older larval stages. Despite this, the number of larvae surviving to adulthood was not affected by any of the treatments; neither was the pupal sex ratio. Exposure of larvae to methyltestosterone significantly increased the number of deformed pupae. In addition, the reproductive potential of adults derived from the testosterone treatment was markedly reduced. Exposure of *L. oleracea* larvae to this steroid caused a high decrease in egg production, coupled with a significant decrease in egg viability.

G. Echinodermata

In the EDIETA report seven studies are cited using echinoderms as test organisms, mainly the starfish *Asterias rubens* (Table VI). To determine endocrine disruption both heavy metals and PCBs, PCP as well as E2, were used as test chemicals.

TABLE VI
Representative Laboratory and Field Studies in Which Endocrine Disruption May Be Occurring in Echinoderms[a]

Species	Contaminant	Concentration (range), effects	Lab/field	Reference
Asterias rubens (adult)	Cadmium	25 µg/liter (acute), 200 µg/liter (chronic); delayed germinal vesicle breakdown during induced spawning	Semifield (acute)/ lab (chronic)	den Besten *et al.*, 1989, 1991a,b
Asterias rubens (adult)	Cadmium, zinc	100 g/liter (cadmium), 240 µg/liter (zinc); increased steroid metabolism, elevated testosterone and progesterone levels	Lab	Voogt *et al.*, 1987
Asterias rubens	PCBs	–; abnormal development of fertilized oocytes, abnormal embryo development, elevated steroid levels in gonads	Semifield	den Besten *et al.*, 1989, 1991a
Asterias rubens	17β-Estradiol	Administered by injection; increased estrone levels in ovaries, increased oocyte diameters	Lab	Schoenmakers *et al.*, 1981
Strongylocentrotus intermedius	Cadmium	0.1–5.0 mg/liter; low fertilization success, formation of anomalous sex cells, arrested embryogenesis	Lab	Khristoforova *et al.*, 1984
Paracentrotus lividus (developing eggs)	Pentachlorophenol	0.1–0.8 mg/liter; alteration in embryonic development and differentiation, reduced echinochrome synthesis	Lab	Ozretic and Krajnovic-Ozretic, 1985
Sclerasterias mollis (adults)	17β-Estradiol, estrone	Daily injections; increased ovarian estrone and progesterone levels	Lab	Barker and Xu, 1993

[a]Modified from deFur *et al.* (1999).

Echinoderm regeneration following autotomy is an important asexual mechanism of reproduction and largely under endocrine control. Therefore, it provides a convenient and tractable test to monitor the effects of endocrine-active chemicals on the developmental physiology (tissue and cellular level) of marine animals (Carnevali *et al.*, 2001a,b). The crinoid *Antedon mediterranea* is especially sensitive to PCBs. For example, exposure to low concentrations of Aroclor 1260 (14 ng/liter of total PCBs) results in abnormal arm growth in terms of both gross morphology and microscopic anatomy (Carnevali *et al.*, 2001a). In terms of tissue/cellular aspects, the main modifications are an accelerated growth of the regenerate with a massive cell migration and proliferation. Furthermore, a hypertrophic development of the coelomic canals and an extensive rearrangement of differentiated tissues of the stump were assessed. The anomalies observed in the developmental regenerative processes appear to be compatible with a pattern of pseudoendocrine activities.

H. Tunicata

Only one publication was found concerning effects of endocrine disrupters on the phylum Tunicata indicating that TBT may act as an endocrine disrupter in ascidians. Patricolo *et al.* (2001) investigated the effects of TBT (up to 10^{-5} *M*) on the metamorphosis of ascidian larvae of *Ciona intestinalis* 2 hours after hatching. A blocked metamorphosis and reduced thyroxin production were noticed in TBT-exposed larvae by the authors.

V. Conclusions and Future Prospects

There is strong evidence, provided by numerous case studies, for effects on development, fecundity, and reproductive output of invertebrates, which can be attributed to substances acting as EDCs. However, there are also some cases where the evidence of a causal link with endocrine disruption is rather weak. Although an increasing number of research projects investigated ED in invertebrates worldwide and the published examples of potential effects of EDCs in these groups have increased in the years since the EDIETA workshop by almost 60%, the main conclusions as summarized by deFur *et al.* (1999) are still valid. With the exception of TBT effects in mollusks, which have been associated with a locally severe impact on community levels, and IGRs in terrestrial insects, there are only a few field examples of ED in invertebrates. Nevertheless, many more examples for ED affecting invertebrate populations and communities can be expected, though still undetected. This assumption is supported by a number of indications:

- Chemical signaling systems and their basic mechanisms in the animal kingdom exhibit a considerable degree of conservatism (McLachlan, 2001). Consequently, invertebrate endocrine function should be affected by identical or similar compounds as vertebrates (deFur *et al.*, 1999; Pinder and Pottinger, 1999).
- Highly effective EDCs have been intentionally developed for the purpose of pest control to interfere with hormonal systems of insects. Such endocrine-mediating properties can be assumed not to be unique for the IGRs or this group of arthropods but rather reflect the fact that much less research has been undertaken for invertebrate groups other than insects.
- ED in invertebrates received far less attention than in vertebrates in the past, probably because their hormonal systems are poorly understood, favoring investigations with vertebrates and especially fish as systematic groups for ecotoxicological research and routine analyses with which many scientists feel familiar.

However, on the basis of the publications on ED in invertebrates presented in this paper, the following general observations should be emphasized:

- Little work has been done on endocrine disruption in invertebrates from the field (with the exception of the investigation of the imposex phenomenon in marine gastropods). The overwhelming majority of laboratory-based studies (56 reports) focus on mollusks (17), crustaceans (15), and insects (12), thus continuing the main tendencies in the pre-1999 literature (Fig. 4a and b).
- Endocrine-mediated effects of xenobiotics in terrestrial species still constitute less than 10% of all reports (Fig. 4c and d).
- Single studies on ED in taxa formerly not considered are available now (e.g., for Porifera, Rotatoria, Tunicata).
- Marine environments and invertebrates have received comparatively little attention (except for the investigation of the imposex phenomenon in marine gastropods).

Our unawareness of invertebrate endocrinology is probably the most important reason for the unsatisfactory progress made regarding ED in invertebrates in the past. Furthermore, 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. This makes the general conditions for research with invertebrates less favorable. Nevertheless, consideration of invertebrates in such research programs potentially offers a wealth of knowledge in understanding comparative and ecological aspects of ED (deFur *et al.*, 1999). For these reasons, invertebrates should

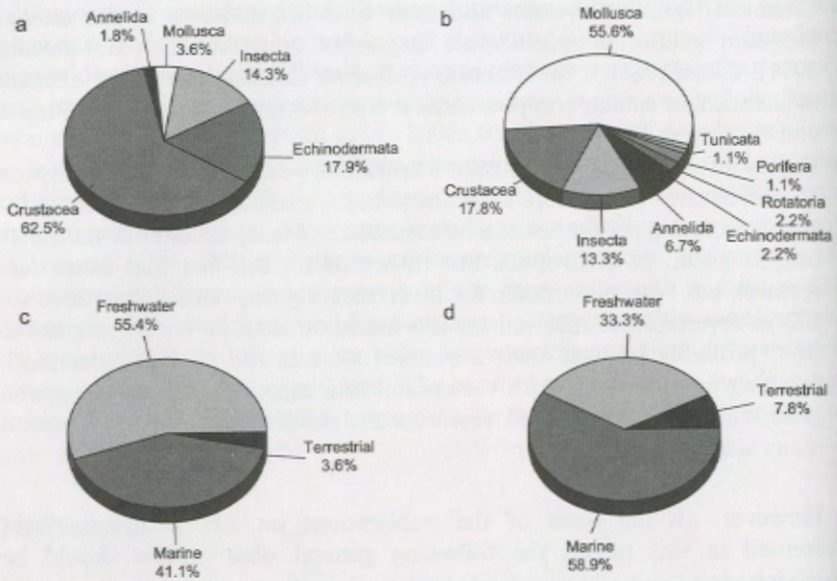

FIG. 4 Overview on the analyzed systematic groups of invertebrates presented in the proceedings of the EDIETA workshop in 1998 (a) (56 studies) and since 1998 (b) (90 studies) as well as the portion of freshwater, marine, and terrestrial species used for ED studies up to 1998 (c) and since 1998 (d).

have a high priority for further research, especially for the development of EDC screening and testing methods. It will be essential to conduct ecological monitoring techniques and to perform more basic research on invertebrate endocrinology, especially on systematic groups, which have been almost totally neglected so far (e.g., Cnidaria, Plathelminthes, and Nematoda, with approximately 55,000 known species worldwide). Hormone receptors of invertebrates should be identified, cloned, and characterized facilitating the identification of receptors that are shared by different groups and finally opening possibilities for the development of extrapolation techniques for effects between species. Additionally, this would help to develop receptor-binding assays and other *in vitro* systems as a screening tool.

A further requirement should be the characterization on endocrine control of toxicological end points in tests in more depth so that these end points can be used as valid measures for ED. New invertebrate tests with endocrine-regulated end points 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 on valid endocrine-mediated end points in invertebrates is too preliminary to design specific monitoring programs for biological effects of EDCs, perhaps with the exception of androgenic and estrogenic compounds and their effects in prosobranch snails. Nevertheless, carefully targeted monitoring programs are needed because effects in invertebrates are probably widespread but undetected.

References

Albalat, A., Potrykus, J., Pempkowiak, J., and Porte, C. (2002). Assessment of organotin pollution along the Polish coast (Baltic Sea) by using mussels and fish as sentinel organisms. *Chemosphere* **47,** 165–171.

Alzieu, C., Thibaud, Y., Héral, M., and Boutier, B. (1980). Évaluation des risques dus a l'emploi des peintures anti-salissures dans les zones conchylicoles. *Rev. Trav. Inst. Pêch. Mar.* **44,** 306–348.

Axiak, V., Micallef, D., Muscat, J., Vella, A., and Mintoff, B. (2003). Imposex as a biomonitoring tool for marine pollution by tributyltin: Some further observations. *Environ. Int.* **28,** 743–749.

Bacchetta, R., Manteca, P., and Vailati, G. (2002). Oocyte degeneration and altered ovipository activity induced by paraquat in the freshwater snail *Physa fontinalis* (Gastropoda: Pulmonata). *J. Molluscan Stud.* **68,** 181–186.

Baldwin, W. S., Milam, D. L., and LeBlanc, G. A. (1995). Physiological and biochemical perturbations in *Daphnia magna* following exposure to the model environmental estrogen diethylstilbestrol. *Environ. Toxicol. Chem.* **14,** 945–952.

Baldwin, W. S., Graham, S. E., Shea, D., and LeBlanc, G. A. (1997). Metabolic androgenization of female *Daphnia magna* by the xenoestrogen 4-nonylphenol. *Environ. Toxicol. Chem.* **16,** 1905–1911.

Baldwin, W. S., Bailey, R., Long, K. E., and Klaine, S. (2001). Incomplete ecdysis is an indicator of ecdysteroid exposure in *Daphnia magna*. *Environ. Toxicol. Chem.* **20,** 1564–1569.

Barker, M. F., and Xu, R. A. (1993). Effects of estrogens on gametogenesis and steroid levels in the ovaries and pyloric cacea of *Sclerasterias mollis* (Echinodermata: Asteroidea). *Invert. Reprod. Dev.* **24,** 53–58.

Barreiro, R., Gonzalez, R., Quintela, M., and Ruiz, J. M. (2001). Imposex, organotin bioaccumulation and sterility of female *Nassarius reticulatus* in polluted areas of NW Spain. *Mar. Ecol. Prog. Ser.* **218,** 203–212.

Barroso, C. M., and Moreira, M. H. (2002). Spatial and temporal changes of TBT pollution along the Portuguese coast: Inefficacy of the EEC directive 89/677. *Mar. Pollut. Bull.* **44,** 480–486.

Barroso, C. M., Moreira, M. H., and Bebianno, M. J. (2002). Imposex, female sterility and organotin contamination of the prosobranch *Nassarius reticulatus* from the Portuguese coast. *Mar. Ecol. Prog. Ser.* **230,** 127–135.

Bauer, B., Fioroni, P., Ide, I., Liebe, S., Oehlmann, J., Stroben, E., and Watermann, B. (1995). TBT effects on the female genital system of *Littorina littorea*: A possible indicator of tributyltin pollution. *Hydrobiologia* **309,** 15–27.

Bech, M. (2002a). A survey of imposex in muricids from 1996 to 2000 and identification of optimal indicators of tributyltin contamination along the east coast of Phuket Island, Thailand. *Mar. Pollut. Bull.* **44,** 887–896.

Bech, M. (2002b). Imposex and tributyltin contamination as a consequence of the establishment of a marina, and increasing yachting activities at Phuket Island, Thailand. *Environ. Pollut.* **117,** 421–429.

Bech, M., Strand, J., and Jacobsen, J. A. (2002). Development of imposex and accumulation of butyltin in the tropical muricid *Thais distinguenda* transplanted to a TBT contaminated site. *Environ. Pollut.* **119,** 253–260.

Beckmann, M., Hardege, J. D., and Zeeck, E. (1995). Effects of the volatile fraction of crude-oil on spawning behavior of nereids (Annelida, Polychaeta). *Mar. Environ. Res.* **40,** 267–276.

Bettin, C., Oehlmann, J., and Stroben, E. (1996). TBT-induced imposex in marine neogastropods is mediated by an increasing androgen level. *Helgoländer Meeresunters.* **50,** 299–317.

Billinghurst, Z., Clare, A. S., Matsumura, K., and Depledge, M. H. (2000). Induction of cypris major protein in barnacle larvae by exposure to 4-n-nonylphenol and 17β-oestradiol. *Aquat. Toxicol.* **47,** 203–212.

Billinghurst, Z., Clare, A. S., and Depledge, M. H. (2001). Effects of 4-n-nonylphenol and 17β-oestradiol on early development of the barnacle *Elminius modestus*. *J. Exp. Mar. Biol. Ecol.* **257,** 255–268.

Binelli, A., and Provini, A. (2003). DDT is still a problem in developed countries: The heavy pollution of Lake Maggiore. *Chemosphere* **52,** 717–723.

Binelli, A., Bacchetta, R., Vailati, G., Galassi, S., and Provini, A. (2001). DDT contamination in Lake Maggiore (N. Italy) and effects on zebra mussel spawning. *Chemosphere* **45,** 409–415.

Birchenough, A. C., Evans, S. M., Moss, C., and Welch, R. (2002a). Re-colonisation and recovery of populations of dogwhelks *Nucella lapillus* (L.) on shores formerly subject to severe TBT contamination. *Mar. Pollut. Bull.* **44,** 652–659.

Birchenough, A. C., Barnes, N., Evans, S. M., Hinz, H., Kronke, I., and Moss, C. (2002b). A review and assessment of tributyltin contamination in the North Sea, based on surveys of butyltin tissue burdens and imposex/intersex in four species of neogastropods. *Mar. Pollut. Bull.* **44,** 534–543.

Blaise, C., Gagne, F., Pellerin, J., and Hansen, P. D. (1999). Determination of vitellogenin-like properties in *Mya arenaria* hemolymph (Saguenay Fjord, Canada): A potential biomarker for endocrine disruption. *Environ. Toxicol.* **14,** 455–465.

Bodar, C. W. M., Vandersluis, I., Voogt, P. A., and Zandee, D. I. (1988). Effects of cadmium on consumption, assimilation and biochemical parameters of *Daphnia magna*—possible implications for reproduction. *Comp. Biochem. Phys. C* **90,** 341–346.

Bodar, C. W. M., Voogt, P. A., and Zandee, D. I. (1990). Ecdysteroids in *Daphnia magna*—their role in molting and reproduction and their levels upon exposure to cadmium. *Aquat. Toxicol.* **17,** 339–350.

Breitholtz, M., and Bengtsson, B. E. (2001). Oestrogens have no hormonal effect on the development and reproduction of the harpacticoid copepod *Nitocra spinipes*. *Mar. Pollut. Bull.* **42,** 879–886.

Breitholtz, M., and Wollenberger, L. (2003). Effects of three PBDEs on development, reproduction and population growth rate of the harpacticoid copepod *Nitocra spinipes*. *Aquat. Toxicol.* **64,** 85–96.

Brown, D., Thompson, R. S., Stewart, K. M., Croudace, C. P., and Gillings, E. (1996). The effect of phthalate ester plasticisers on the emergence of the midge (*Chironomus riparius*) from treated sediments. *Chemosphere* **32,** 2177–2187.

Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., and Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the *Daphnia magna*. *Chemosphere* **36,** 1367–1379.

Brown, R. J., Conradi, M., and Depledge, M. H. (1999). Long-term exposure to 4-nonylphenol affects sexual differentiation and growth of the amphipod *Corophium volutator* (Pallas, 1766). *Sci. Total Environ.* **233,** 77–88.

Bryan, G. W., and Gibbs, P. E. (1991). Impact of low concentrations of tributyltin (TBT) on marine organisms: A review. *In* "Metal Ecotoxicology: Concepts and Applications" (M. C. Newman and A. W. McIntosh, Eds.), pp. 323–361. Lewis Publisher, Ann Arbor.

Carnevali, M. D. C., Bonasoro, F., Patrono, M., Thorndyke, M. C., and Galassi, S. (2001a). PCB exposure and regeneration in crinoids (Echinodermata). *Mar. Ecol. Prog. Ser.* **215,** 155–167.

Carnevali, M. D. C., Galassi, S., Bonasoro, F., Patrono, M., and Thorndyke, M. C. (2001b). Regenerative response and endocrine disrupters in crinoid echinoderms: Arm regeneration in *Antedon mediterranea* after experimental exposure to polychlorinated biphenyls. *J. Exp. Biol.* **204,** 835–842.

Celestial, D. M., and McKennedy, C. L. (1994). The influence of an insect growth regulator on the larval development of the mud crab *Rhithropanopeus harrisii*. *Environ. Pollut.* **85,** 169–173.

Chiavarini, S., Massanisso, P., Nicolai, P., Nobili, C., and Morabito, R. (2003). Butyltins concentration levels and imposex occurrence in snails from the Sicilian coasts (Italy). *Chemosphere* **50,** 311–319.

Coelho, M. R., Fuentes, S., and Bebianno, M. J. (2001). TBT effects on the larvae of *Ruditapes decussates*. *J. Mar. Biol. Assoc. UK* **81,** 259–265.

Coelho, M. R., Bebianno, M. J., and Langston, W. J. (2002). Organotin levels in the Ria Formosa lagoon, Portugal. *Appl. Organomet. Chem.* **16,** 384–390.

Comber, M. H. I., Williams, T. D., and Stewart, K. M. (1993). The effects of nonylphenol on *Daphnia magna*. *Water Res.* **27,** 273–276.

Daniels, R. E., and Allan, J. D. (1981). Life table evaluation of chronic exposure to a pesticide. *Can. J. Fish. Aquat. Sci.* **38,** 485–494.

de Bisthoven, L. J., Huysmans, C., and Ollevier, F. (1995). The *in situ* relationships between sediment concentrations of micropollutants and morphological deformities in *Chironomus thummi* larvae (Diptera, Chironomidae) from lowland rivers (Belgium): A spatial comparision. *In* "Chironomids: From Genes to Ecosystems" (P. S. Cranston, Ed.), pp. 63–80. Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra.

Dedos, S. G., Szurdoki, F., Szekacs, A., Shiotsuki, T., Hammock, B. D., Shimada, J., and Fugo, H. (2002a). Fenoxycarb levels and their effects on general and juvenile hormone esterase activity in the hemolymph of the silkworm, *Bombyx mori* Pest. *Biochem. Phys.* **73,** 174–187.

Dedos, S. G., Szurdoki, F., Szekacs, A., Mizoguchi, A., and Fugo, H. (2002b). Induction of dauer pupae by fenoxycarb in the silkworm, *Bombyx mori*. *J. Insect Physiol.* **48,** 857–865.

deFur, P. L., Crane, M., Ingersoll, C., and Tattersfield, L. (Eds.) (1999). *In* "Endocrine Disruption in Invertebrates: Endocrinology, Testing, and Assessment. Proceedings of the Workshops on Endocrine Disruption in Invertebrates, 12–15 December 1998, Noordwijkerhout, The Netherlands." SETAC Press, Pensacola.

den Besten, P. J., Herwig, H. J., Zandee, D. I., and Voogt, P. A. (1989). Effects of cadmium and PCBs on reproduction of the seastar *Asterias rubens*: Aberrations in the early development. *Ecotoxicol. Environ. Saf.* **18,** 173–180.

den Besten, P. J., Elenbaas, J. M. L., Maas, J. R., Dieleman, S. J., Herwig, H. J., and Voogt, P. A. (1991a). Effects of cadmium and polychlorinated biphenyls (Clophen A50) on steroid

metabolism and cytochrome P-450 monooxygenase system in the sea star *Asterias rubens* L. *Aquat. Toxicol.* **20,** 95–110.

den Besten, P. J., van Donselaar, Herwig, H. J., Zandee, D. I., and Voogt, P. A. (1991b). Effects of cadmium on gametogenesis in sea star *Asterias rubens* L. *Aquat. Toxicol.* **20,** 83–94.

Depledge, M. H., and Billinghurst, Z. (1999). Ecological significance of endocrine disruption in marine invertebrates. *Mar. Pollut. Bull.* **39,** 32–38.

Dhadialla, T. S., Carlson, R. R., and Le, D. P. (1998). New insecticides with ecdysteroidal and juvenile hormone activity. *Annu. Rev. Entomol.* **43,** 545–569.

Dickman, M., and Rygiel, G. (1996). Chironomid larval deformity frequencies, mortality, and diversity in heavy-metal contaminated sediments of a Canadian Riverine Wetland. *Environ. Int.* **22,** 693–703.

Dinan, L., Bourne, P., Whiting, P., Dhadialla, T. S., and Hutchinson, T. H. (2001). Screening of environmental contaminants for ecdysteroid agonist and antagonist activity using the *Drosophila melanogaster* B-II cell in vitro assay. *Environ. Toxicol. Chem.* **20,** 2038–2046.

Dorn, A. (Ed.) (2000). *In* "Reproductive Biology of Invertebrates," Vol. 10, Part A and B. John Wiley & Sons, New York.

Duft, M., Schulte-Oehlmann, U., Tillmann, M., Markert, B., and Oehlmann, J. (2003a). Toxicity of triphenyltin and tributyltin to the freshwater mudsnail *Potamopyrgus antipodarum* in a new sediment biotest. *Environ. Toxicol. Chem.* **22,** 145–152.

Duft, M., Schulte-Oehlmann, U., Weltje, L., Tillmann, M., and Oehlmann, J. (2003b). Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail *Potamopyrgus antipodarum*. *Aquat. Toxicol.* **64,** 437–449.

Evans, S. M., Bech, M., Hawkins, S. T., Smith, R., and Stewart, A. (2001). Imposex in populations of dogwhelks *Thais* spp. in relation to shipping intensity and mariculture activity in East Asia. *Invert. Reprod. Dev.* **39,** 231–237.

Féral, C., and Le Gall, S. (1982). Induction expérimentale par un polluant marin (le tributylétain), de l'activité neuroendocrine contrôlant la morphogenèse du pénis chez les femelles *d'Ocenebra erinacea* (Mollusque Prosobranche gonochorique). *C. R. Acad. Sci. Paris* **295,** Série III, 627–630.

Fernandez-Casalderry, A., Ferrando, M. D., and Andreumoliner, E. (1993). Effects of endosulfan on survival, growth and reproduction of *Daphnia magna*. *Comp. Biochem. Phys. C* **106,** 437–441.

Fernandez-Casalderry, A., Ferrando, M. D., and Andreumoliner, E. (1994). Effect of sublethal concentrations on the feeding-behavior of *Daphnia magna*. *Ecotox. Environ. Saf.* **27,** 82–89.

Fingerman, S. W., and Fingerman, M. (1978). Influence of polychlorinated biphenyl preparation Arochlor 1242 on color changes of the fiddler crab *Uca pugilator*. *Mar. Biol.* **50,** 37–45.

Fioroni, P., Oehlmann, J., and Stroben, E. (1991). The pseudohermaphroditism of prosobranchs; morphological aspects. *Zool. Anz.* **226,** 1–26.

Fitzmayer, K. M., Geiger, J. G., and Vandenavyle, M. J. (1982). Effects of chronic exposure to Simazine on the cladoceran, *Daphnia pulex*. *Arch. Environ. Contam. Toxicol.* **11,** 603–609.

Følsvik, N., Berge, J. A., Brevik, E. M., and Walday, M. (1999). Quantification of organotin compounds and determination of imposex in populations of dogwhelks (*Nucella lapillus*) from Norway. *Chemosphere* **38,** 681–691.

Gagne, F., Blaise, C., Pellerin, J., Pelletier, E., Douville, M., Gauthier-Clerc, S., and Viglino, L. (2003). Sex alteration in soft-shell clams (*Mya arenaria*) in an intertidal zone of the Saint Lawrence River (Quebec, Canada). *Comp. Biochem. Phys. C* **134,** 189–198.

Gauthier-Clerc, S., Pellerin, J., Blaise, C., and Gagne, F. (2002). Delayed gametogenesis of *Mya arenaria* in the Saguenay fjord (Canada): A consequence of endocrine disruptors? *Comp. Biochem. Physiol. C* **131,** 457–467.

Gibbs, P. E., Bryan, G. W., Pascoe, P. L., and Burt, G. R. (1987). The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. *J. Mar. Biol. Assoc. UK.* **67,** 507–523.

Gibson, C. P., and Wilson, S. P. (2003). Imposex still evident in eastern Australia 10 years after tributyltin restrictions. *Mar. Environ. Res.* **55,** 101–112.

Gooding, M. P., Wilson, V. S., Folmar, L. C., Marcovich, D. T., and LeBlanc, G. A. (2003). The biocide tributyltin reduces the accumulation of testosterone as fatty acid esters in the mud snail (*Ilyanassa obsoleta*). *Environ. Health Perspect.* **111,** 426–430.

Gorbman, A., and Davey, K. (1991). Endocrines. *In* "Neureal and Integrative Animal Physiology" (C. L. Prosser, Ed.), pp. 693–754. Wiley-Liss, New York.

Gross, M. Y., Maycock, D. S., Thorndyke, M. C., Morritt, D., and Crane, M. (2001). Abnormalities in sexual development of the amphipod *Gammarus pulex* (L.) found below sewage treatment works. *Environ. Toxicol. Chem.* **20,** 1792–1797.

Gross-Sorokin, M. Y., Grist, E. P. M., Cooke, M., and Crane, M. (2003). Uptake and depuration of 4-nonylphenol by the benthic invertebrate *Gammarus pulex*: How important is feeding rate? *Environ. Sci. Technol.* **37,** 2236–2241.

Hagger, J. A., Fisher, A. S., Hill, S. J., Depledge, M. H., and Jha, A. N. (2002). Genotoxic, cytotoxic and ontogenetic effects of tri-n-butyltin on the marine worm, *Platynereis dumerilii* (Polychaeta: Nereidae). *Aquat. Toxicol.* **57,** 243–255.

Hahn, T., and Schulz, R. (2002). Ecdysteroid synthesis and imaginal disc development in the midge *Chironomus riparius* as biomarkers for endocrine effects of tributyltin. *Environ. Toxicol. Chem.* **21,** 1052–1057.

Hahn, T., Liess, M., and Schulz, R. (2001). Effects of the hormone mimetic insecticide tebufenozide on *Chironomus riparius* larvae in two different exposure setups. *Ecotox. Environ. Saf.* **49,** 171–178.

Hahn, T., Schenk, K., and Schulz, R. (2002). Environmental chemicals with known endocrine potential affect yolk protein content in the aquatic insect *Chironomus riparius*. *Environ. Pollut.* **120,** 525–528.

Hamilton, A. L., and Seather, O. A. (1971). The occurrence of characteristic deformities in the chironomid larvae of several Canadian lakes. *Can. Entomol.* **103,** 363–368.

Hanumante, M. M., Fingerman, S. W., and Fingerman, M. (1981). Antagonism of the inhibitory effect of the polychlorinated biphenyl preparation, Arochlor-1242, on color changes of the fiddler crab, *Uca pugilator*, by norepinephrine and drugs affecting noradrenergic neurotransmission. *Bull. Environ. Contam. Toxicol.* **26,** 479–484.

Hill, M., Stabile, C., Steffen, L. K., and Hill, A. (2002). Toxic effects of endocrine disrupters on freshwater sponges: Common developmental abnormalities. *Environ. Pollut.* **117,** 295–300.

Hines, G. A., Watts, S. A., Sower, S. A., and Walker, C. W. (1992). Sex steroid levels in the testis, ovaries, and poloric ceca during gametogenesis in the sea star *Asterias vulgaris*. *Gen. Comp. Endocrin.* **87,** 451–460.

Hoffmann, J., and Porchet, M. (Eds.) (1984). "Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones." Springer-Verlag, New York.

Horiguchi, T., Shiraishi, H., Shimizu, M., and Morita, M. (1997). Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, *Thais clavigera*. *Environ. Pollut.* **95,** 85–91.

Horiguchi, T., Takiguchi, N., Cho, H. S., Kojima, M., Kaya, M., Shiraishi, H., Morita, M., Hirose, H., and Shimizu, M. (2000). Ovo-testis and disturbed reproductive cycle in the giant abalone, *Haliotis madaka*: Possible linkage with organotin contamination in a site of population decline. *Mar. Environ. Res.* **50,** 223–229.

Horiguchi, T., Kojima, M., Kaya, M., Matsuo, T., Shiraishi, H., Morita, M., and Adachi, Y. (2002). Tributyltin and triphenyltin induce spermatogenesis in ovary of female abalone, *Haliotis gigantean*. *Mar. Environ. Res.* **54,** 679–684.

Horiguchi, T., Li, Z., Uno, S., Shimizu, M., Shiraishi, H., Morita, M., Thompson, J. A. J., and Levings, C. D. (2004). Contamination of organotin compounds and imposex in molluscs from Vancouver, Canada. *Mar. Environ. Res.* **57,** 75–88.

Hung, T. C., Hsu, W. K., Mang, P. J., and Chuang, A. (2001). Organotins and imposer in the rock shell, *Thais clavigera*, from oyster mariculture areas in Taiwan. *Environ. Pollut.* **112,** 145–152.

Ide, I., Witten, E. P., Fischer, J., Kalbfus, W., Zellner, A., Stroben, E., and Watermann, B. (1997). Accumulation of organotin compounds in the common whelk *Buccinum undatum* and the red whelk *Neptunea antiqua* in association with imposex. *Mar. Ecol. Prog. Ser.* **152,** 197–203.

Ishaaya, I. (1992). Insect resistance to benzylphenylureas and other insect growth regulators. *In* "Molecular Mechanism of Insecticide Resistance: Diversity Amongst Insects" (C. A. Mullin and J. G Scott, Eds.), ACS Symposium Series 205, pp. 231–246. American Chemical Society, Washington, D.C.

Jha, A. N., Hagger, J. A., Hill, S. J., and Depledge, M. H. (2000a). Genotoxic, cytotoxic and developmental effects of tributyltin oxide (TBTO): An integrated approach to the evaluation of the relative sensitivities of two marine species. *Mar. Environ. Res.* **50,** 565–573.

Jha, A. N., Hagger, J. A., and Hill, S. J. (2000b). Tributyltin induces cytogenetic damage in the early life stages of the marine mussel, *Mytilus edulis*. *Environ. Mol. Mutagen.* **35,** 343–350.

Johnston, P. A. (1987). Acute toxicity of inorganic selenium to *Daphnia magna* (Straus) and the effect of subacte exposure upon growth and reproduction. *Aquat. Toxicol.* **10,** 335–352.

Kahl, M. D., Makynen, E. A., Kosian, P. A., and Ankley, G. T. (1997). Toxicity of 4-nonylphenol in a life-cycle test with the migde *Chironomus tentans*. *Ecotoxicol. Environ. Saf.* **38,** 155–160.

Kersting, K. (1975). The use of microsystems for the evaluation of the effect of toxicants. *Hydrobiol. Bull.* **9,** 102–108.

Khristoforova, N. K., Gnezdilova, S. M., and Vlasova, G. A. (1984). Effects of cadmium on gametogenesis and offspring of the sea urchin *Strongylocentrotus intermedius*. *Mar. Ecol. Prog. Ser.* **17,** 9–14.

Kirkbride-Smith, A. E., Bell, H. A., and Edwards, J. P. (2001). Effects of three vertebrate hormones on the growth, development and reproduction of the tomato moth, *Lacanobia oleracea* L. (Lepidoptera: Noctuidae). *Environ. Toxicol. Chem.* **20,** 1838–1845.

Kluytmans, J. H., Brands, F., and Zandee, D. I. (1988). Interactions of cadmium with the reproductive-cycle of *Mytilus edulis*. *Mar. Environ. Res.* **24,** 189–192.

Lafont, R. (2000). The endocrinology of invertebrates. *Ecotoxicology* **9,** 41–57.

LeBlanc, G. A., and McLachlan, J. B. (1999). Molt-independent growth inhibition of *Daphnia magna* by a vertebrate antiandrogen. *Environ. Toxicol. Chem.* **18,** 1450–1455.

Matthiessen, P., and Gibbs, P. E. (1998). Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in molluscs. *Environ. Toxicol. Chem.* **17,** 37–43.

Matthiessen, P., and Law, R. J. (2002). Contaminants and their effects on estuarine and coastal organisms in the United Kingdom in the late twentieth century. *Environ. Pollut.* **120,** 739–757.

Matthiessen, P., Waldock, R., Thain, J. E., Waite, M. E., and Scrope-Howe, S. (1995). Changes in periwinkle (*Littorina littorea*) populations following the ban on the TBT-based antifoulings on small boats in the United Kindom. *Ecotoxicol. Environ. Saf.* **30,** 180–194.

McKenney, C. L., and Celestial, D. M. (1996). Modified survival, growth and reproduction in an estuarine mysid (*Mysidopsis bahia*) exposed to a juvenile hormone analogue through a complete life cycle. *Aquat. Toxicol.* **35,** 11–20.

McKenney, C. L., and Matthews, E. (1990). Influence of an insectide-growth regulator on the larval development of an estuarine shrimp. *Environ. Pollut.* **64,** 169–178.

McLachlan, J. A. (2001). Environmental signaling: What embryos and evolution teach us about endocrine disrupting chemicals. *Endocr. Rev.* **22,** 319–341.

Meador, J. P., and Rice, C. A. (2001). Impaired growth in the polychaete *Armandia brevis* exposed to tributyltin in sediment. *Mar. Environ. Res.* **51,** 113–129.

Meregalli, G., and Ollevier, F. (2001). Exposure of *Chironomus riparius* larvae to 17α-ethynylestradiol: Effects on survival and mouthpart deformities. *Sci. Total Environ.* **269,** 157–161.

Meregalli, G., Pluymers, L., and Ollevier, F. (2001). Induction of mouthpart deformities in *Chironomus riparius* larvae exposed to 4-*n*-nonylphenol. *Environ. Pollut.* **111,** 241–246.

Morcillo, Y., and Porte, C. (1999). Evidence of endocrine disruption in the imposex-affected gastropod *Bolinus brandaris*. *Environ. Res.* **81,** 349–354.

Morcillo, Y., Albalat, A., and Porte, C. (1999). Mussels as sentinels of organotin pollution: Bioaccumulation and effects on P450-mediated aromatase activity. *Environ. Toxicol. Chem.* **18,** 1203–1208.

Nagabhushanam, R., and Kulkarni, G. K. (1981). Effect of endogenous testosterone on the androgenic gland and testis of a marine penaid prawn, *Parapenaeopsis hardwickii* (Miers) (Crustacea, Decapoda, Panaeidae). *Aquaculture* **23,** 19–27.

Nagabhushanam, R., Reddy, P. S., and Sarojini, R. (1990). Tributyltin oxide induced alterations in exuvial weight and calcium content of the prawn, *Caridina rajadhari*. *Proc. Indian Acad. Sci.* **99,** 397–400.

Nemanic, T. M., Leskovsek, H., Horvat, M., Vriser, B., and Bolje, A. (2002). Organotin compounds in the marine environment of the Bay of Piran, Northern Adriatic sea. *J. Environ. Monitor.* **4,** 426–430.

Nice, H. E., Throndyke, M. C., Morritt, D., Steele, S., and Crane, M. (2000). Development of *Crassostrea gigas* larvae is affected by 4-nonylphenol. *Mar. Pollut. Bull.* **40,** 491–496.

Nice, H. E., Morritt, D., Crane, M., and Thorndyke, M. (2003). Long-term and transgenerational effects of nonylphenol exposure at a key stage in the development of *Crassostrea gigas*. Possible endocrine disruption? *Mar. Ecol. Prog. Ser.* **256,** 256–300.

Obara, Y., Miyatani, M., Ishiguro, Y., Hirota, K., Koyama, T., Izumi, S., Iwami, M., and Sakurai, S. (2002). Pupal commitment and its hormonal control in wing imaginal discs. *J. Insect Physiol.* **48,** 933–944.

Oberdörster, E., and Cheek, A. O. (2001). Gender benders at the beach: Endocrine disruption in marine and estuarine organisms. *Environ. Toxicol. Chem.* **20,** 23–36.

Oberdörster, E., Rice, C. D., and Irwin, L. K. (2000). Purification of vitellin from grass shrimp *Palaemonetes pugio*, generation of monoclonal antibodies, and validation for the detection of lipovitellin in Crustacea. *Comp. Biochem. Physiol. C.* **127,** 199–207.

Oehlmann, J., and Schulte-Oehlmann, U. (2003). Molluscs as bioindicators. *In* "Bioindicators and Biomonitors—Principles, Concepts and Applications" (B. A. Markert, A. M. Breure, and H. G. Zechmeister, Eds.), pp. 577–635. Elsevier, Amsterdam.

Oehlmann, J., Fioroni, P., Stroben, E., and Markert, B. (1996a). Tributyltin (TBT) effects on *Ocinebrina aciculata* (Gastropoda: Muricidae): Imposex development, sterilization, sex change and population decline. *Sci. Total Environ.* **188,** 205–223.

Oehlmann, J., Stroben, E., Schulte-Oehlmann, U., Bauer, B., Fioroni, P., and Markert, B. (1996b). Tributyltin biomonitoring using prosobranchs as sentinel organisms. *Fres. J. Anal. Chem.* **354,** 540–545.

Oehlmann, J., Schulte-Oehlmann, U., Tillmann, M., and Markert, B. (2000). Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol A and octylphenol as xeno-estrogens. *Ecotoxicology* **9,** 383–397.

Olmstead, A. W., and LeBlanc, G. A. (2000). Effects of endocrine-active chemicals on the development of sex characteristics of *Daphnia magna*. *Environ. Toxicol. Chem.* **19,** 2107–2113.

Olmstead, A. W., and LeBlanc, G. A. (2002). Juvenoid hormone methyl farnesoate is a sex determinant in the crustacean *Daphnia magna*. *J. Exp. Zool.* **293,** 736–739.

Olmstead, A. W., and LeBlanc, G. A. (2003). Insecticidal juvenile hormone analogs stimulate the production of male offspring in the crustacean *Daphnia magna*. *Environ. Health Perspect.* **111,** 919–924.

Ozretic, B., and Krajnovic-Ozretic, M. (1985). Morphological and biochemical evidence of the toxic effect of pentachlorophenol the developing embryos of the sea urchin. *Aquat. Toxicol.* **7,** 255–263.

Oslo and Paris Commissions (Ed.) (1996). "Agenda Item 9 of the Environmental Assessment and Monitoring Committee (ASMO)." OSPAR, Vila Franca (= ASMO 96/9/8-E).

Parks, L. G., and LeBlanc, G. A. (1996). Reductions in steroid hormone biotransformation/elimination as a biomarker of pentachlorophenol chronic toxicity. *Aquat. Toxicol.* **34,** 291–303.

Patricolo, E., Mansueto, C., D'Agati, P., and Pellerito, L. (2001). Organometallic complexes with biological molecules: XVI. Endocrine disruption effects of tributyltin(IV) chloride on metamorphosis of the ascidian larva. *Appl. Organomet. Chem.* **15,** 916–923.

Pinder, L. C. V., and Pottinger, T. G. (1999). "Endocrine Function in Aquatic Invertebrates and Evidence for Disruption by Environmental Pollutants." Centre for Ecology and Hydrology, Natural Environmental Research Council, Ambleside.

Pounds, N. A., Hutchinson, T. H., Williams, T. D., Whiting, P., and Dinan, L. (2002). Assessment of putative endocrine disrupters in an *in vivo* crustacean assay and an *in vitro* insect assay. *Mar. Environ. Res.* **54,** 709–713.

Preston, B. L., Snell, T. W., Robertson, T. L., and Dingmann, B. J. (2000). Use of freshwater rotifer *Brachionus calyciflorus* in screening assay for potential endocrine disruptors. *Environ. Toxicol. Chem.* **19,** 2923–2928.

Radix, P., Severin, G., Schramm, K. W., and Kettrup, A. (2002). Reproduction disturbances of *Brachionus calyciflorus* (rotifer) for the screening of environmental endocrine disrupters. *Chemosphere* **47,** 1097–1101.

Ramasamy, M. S., and Murugan, A. (2002). Imposex in muricid gastropod *Thais biserialis* (Mollusca: Neogastropoda: Muricidae) from Tuticorin harbour, southeast coast of India. *Indian J. Mar. Sci.* **31,** 243–245.

Reddy, P. S., Sarojini, R., and Nagabhushanam, R. (1991). Impact of tributyltin oxide (TBTO) on the limb regeneration of the prawn *Caridina rajadhari*, after exposure to different time intervals of amputation. *J. Tissue Res.* **1,** 35–49.

Reddy, P. S., Nagabhushanam, R., and Sarojini, R. (1992). Retardation of molting in the prawn, *Caridina rajadhari*, exposed to tributyltin oxide (TBTO). *Proc. Natl. Acad. Sci. India* **62B,** 353–356.

Ricketts, H. J., Spurgeon, D. J., Morgan, A. J., and Kille, P. (2003). Identification, isolation and characterisation of vitellogenin from the lumbricid earthworm, *Eisenia fetida*. Poster-Abstract of the 7th International Symposium on Earthworm Ecology, 1–6 September 2002, Cardiff, Wales: 214.

Rilov, C., Gasith, A., Evans, S. M., and Benayahu, Y. (2000). Unregulated use of TBT-based antifouling paints in Israel (eastern Mediterranean): High contamination and imposex levels in two species of marine gastropods. *Mar. Ecol. Prog. Ser.* **192,** 229–238.

Rilov, C., Benayahu, Y., and Gasith, A. (2001). Low abundance and skewed population structure of the whelk *Stramonita haemastoma* along the Israeli Mediterranean coast. *Mar. Ecol. Prog. Ser.* **218,** 189–202.

Santos, M. M., Ten Hallers-Tjabbes, C. C., Santos, A. M., and 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. *J. Sea Res.* **48,** 217–223.

Sarojini, R., Nagabhushanam, R., and Fingerman, M. (1994). A possible neurotransmitter-neuroendocrine mechanism in naphthalene-induced atresia of the ovary of the red swamp crayfish, *Procambrus clarkii*. *Comp. Biochem. Phys. C* **108,** 33–38.

Schober, U., and Lampert, W. (1977). Effects of sublethal concentrations of herbicide atrazin on growth and reproduction of *Daphnia pulex*. *Bull. Environ. Contam. Toxicol.* **17,** 269–277.

Schoenmakers, H. J. N., van Bohemen, Ch. G., and Dielemans, S. J. (1981). Effects of oestradiol-17β on the ovaries of the starfish *Asterias rubens*. *Dev. Growth Differ.* **23,** 125–135.

Schulte-Oehlmann, U., Bettin, C., Fioroni, P., Oehlmann, J., and Stroben, E. (1995). *Marisa cornuarietis* (Gastropoda, Prosobranchia): A potential TBT bioindicator for freshwater environments. *Ecotoxicology* **4,** 372–384.

Schulte-Oehlmann, U., Tillmann, M., Markert, B., Oehlmann, J., Watermann, B., and Scherf, S. (2000). Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part II: Triphenyltin as a xeno-androgen. *Ecotoxicology* **9,** 399–412.

Schulte-Oehlmann, U., Tillmann, M., Casey, D., Duft, M., Markert, B., and Oehlmann, J. (2001a). östrogenartige Wirkungen von Bisphenol A auf Vorderkiemerschnecken (Mollusca: Gastropoda: Prosobranchia). *UWSF Z. Umweltchem. ökotox.* **13,** 319–333.

Schulte-Oehlmann, U., Duft, M., Tillmann, M., Markert, B., Stachel, B., and Oehlmann, J. (2001b). "Biological Effects Monitoring with River Elbe Sediments Using *Potamopyrgus antipodarum* and *Hinia (Nassarius) reticulata* (Gastropoda: Prosobranchia)." ARGE Elbe, Hamburg.

Shurin, J. B., and Dodson, S. I. (1997). Sublethal toxic effects of cyanobacteria and nonylphenol on environmental sex determination and development in *Daphnia*. *Environ. Toxicol. Chem.* **16,** 1269–1276.

Smith, B. S. (1971). Sexuality in the American mud snail, *Nassarius obsoletus* Say. *Proc. Malacol. Soc. London* **39,** 377–378.

Staub, G. C., and Fingerman, M. (1984). Effect of naphthalene on color changes of the sand fiddler crab, *Uca pugilator*. *Comp. Biochem. Phys.* **77,** 7–12.

St. Jean, S. D., Pelletier, E., and Courtenay, S. C. (2002a). Very low levels of waterborne butyltins modulate hemocyte function in the blue mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* **236,** 155–161.

St. Jean, S. D., Pelletier, E., and Courtenay, S. C. (2002b). Hemocyte functions and bacterial clearance affected *in vivo* by TBT and DBT in the blue mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* **236,** 163–178.

Strand, J., and Asmund, G. (2003). Tributyltin accumulation and effects in marine molluscs from West Greenland. *Environ. Pollut.* **123,** 31–37.

Strand, J., Jacobsen, J. A., Pedersen, B., and Granmo, A. (2003). Butyltin compounds in sediment and molluscs from the shipping strait between Denmark and Sweden. *Environ. Pollut.* **124,** 7–15.

Subramanian, M. A., and Varadaraj, G. (1993). The effect of industrial effluents on molting in *Macromia cingulata* (Rambur) (Anisoptera: Corduliidae). *Odonatologica* **22,** 229–232.

Sudaryanto, A., Takahashi, S., Monirith, I., Ismail, A., Muchtar, M., Zheng, J., Richardson, B. J., Subramanian, M. A., Prudente, M., Hue, N. D., and Tanabe, S. (2002). Asia-Pacific mussel watch: Monitoring of butyltin contamination in coastal waters of Asian developing countries. *Environ. Toxicol. Chem.* **21,** 2119–2130.

Takeda, N. (2000). Development of a penis from the vestigial penis in the female apple snail, *Pomacea canaliculata*. *Biol. Bull.* **199,** 316–320.

Tatarazako, N., Takao, Y., Kishi, K., Onikura, N., Arizono, K., and Iguchi, T. (2002). Styrene dimers and trimers affect reproduction of daphnid (*Ceriodaphnia dubia*). *Chemosphere* **48,** 597–601.

Ten Hallers-Tjabbes, C. C., Wegener, J. W., Van Hattum, B., Kemp, J. F., Ten Hallers, E., Reitsema, T. J., and Boon, J. P. (2003). Imposex and organotin concentrations in *Buccinum undatum* and *Neptunea antiqua* from the North Sea: Relationship to shipping density and hydrographical conditions. *Mar. Environ. Res.* **55,** 203–233.

Tewari, A., Raghunathan, C., Joshi, H. V., and Khambhaty, Y. (2002). Imposex in rock whelks *Thais* and *Ocenebra* species (Mollusca, Neogastropoda, Muricidae) from Gujarat coast. *Indian J. Mar. Sci.* **31,** 321–328.

Thornton, J. W., Need, E., and Crews, D. (2003). Resurrecting the ancestral steroid receptor: Ancient origin of estrogen signaling. *Science* **301,** 1714–1717.

Tillmann, M., Schulte-Oehlmann, U., Duft, M., Markert, B., and Oehlmann, J. (2001). Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part III: Cyproterone acetate and vinclozoline as antiandrogens. *Ecotoxicology* **10,** 373–388.

Vermeulen, A. C., Liberloo, G., Dumont, P., Ollevier, F., and Goddeeris, B. (2000). Exposure of *Chironomus riparius* larvae (Diptera) to lead, mercury and β-sitosterol: Effects on mouthpart deformation and molting. *Chemosphere* **41,** 1581–1591.

Voogt, P. A., den Besten, P. J., Kusters, C. G. M., and Messing, M. W. J. (1987). Effects of cadmium and zinc on steroid metabolism and steroid levels in the sea star *Asterias rubens* L. *Comp. Biochem. Physiol. C* **86,** 83–89.

Waldock, R., Rees, H. L., Matthiessen, P., and Pendle, M. A. (1999). Surveys of the benthic infauna of the Crouch Estuary (UK) in relation to TBT contamination. *J. Mar. Biol. Assoc. UK* **79,** 225–232.

Watts, M. M., Pascoe, D., and Carroll, K. (2001a). Chronic exposure to 17α-ethinylestradiol and bisphenol A effects on development and reproduction in the freshwater invertebrate *Chironomus riparius* (Diptera: Chironomidae). *Aquat. Toxicol.* **55,** 113–124.

Watts, M. M., Pascoe, D., and Carroll, K. (2001b). Survival and precopulatory behaviour of *Gammarus pulex* (L.) exposed to two xenoestrogens. *Water Res.* **35,** 2347–2352.

Weis, J. S. (1976). The effect of mercury, cadmium and lead salts on limb regeneration in the fiddler crap, *Uca pugilator*. *Fishery Bull.* **74,** 464–467.

Weis, J. S. (1980). Effect of zinc on regeneration in the fiddler crap *Uca pugilator* and its interactions with methylmercury and cadmium. *Mar. Environ. Res.* **3,** 249–255.

Weis, J. S., Gottlieb, J., and Kwiatkowski, J. (1987). Tributyltin retards regeneration and produces deformities of limbs in the fiddler crab, *Uca pugilator*. *Arch. Environ. Contam. Toxicol.* **16,** 321–326.

Widarto, T. H., Forbes, V. E., and Holmstrup, M. (2003). The life-history of an earthworm *Dendrobaena octaedra* under the influence of nonylphenol. Poster-Abstract of the 7th International Symposium on Earthworm Ecology, 1–6 September 2002. Cardiff, Wales: 95.

Woin, P., and Bronmark, C. (1992). Effects of DDT and MCPA (4-chloro-2-methylphenoxyacetic acid) on reproduction of the pond snail *Lynaea stagnalis*. *Bull. Environ. Contam. Toxicol.* **48,** 7–13.

Zou, E., and Fingerman, M. (1997a). Synthetic estrogenic agents do not interfere with sex differentiation but do inhibit molting of the cladoceran *Daphnia magna*. *Bull. Environ. Contam. Toxicol.* **58,** 596–602.

Zou, E., and Fingerman, M. (1997b). Effects of estrogenic xenobiotics on molting of the water flea, *Daphnia magna*. *Ecotox. Environ. Saf.* **38,** 281–285.