COMPRENDO: Focus and Approach

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Tens of thousands of man-made chemicals are in regular use and discharged into the environment. Many of them are known to interfere with the hormonal systems in humans and wildlife. Given the complexity of endocrine systems, there are many ways in which endocrine-disrupting chemicals (EDCs) can affect the body's signaling system, and this makes unraveling the mechanisms of action of these chemicals difficult. A major concern is that some of these EDCs appear to be biologically active at extremely low concentrations. There is growing evidence to indicate that the guiding principle of traditional toxicology that "the dose makes the poison" may not always be the case because some EDCs do not induce the classical dose–response relationships. The European Union project COMPRENDO (Comparative Research on Endocrine Disrupters—Phylogenetic Approach and Common Principles focussing on Androgenic/Antiandrogenic Compounds) therefore aims to develop an understanding of potential health problems posed by androgenic and antiandrogenic compounds (AACs) to wildlife and humans by focusing on the commonalities and differences in responses to AACs across the animal kingdom (from invertebrates to vertebrates). *Key words:* **androgens, antiandrogens, endocrine disruptor, environmental health, molecular screening, phylogenetic approach, wildlife exposure.** *Environ Health Perspect* **114(suppl 1):98–100 (2006). doi:10.1289/ehp.8060 available via** *http://dx.doi.org/* **[Online 30 March 2006]**

Endocrine Disruptors: The Issue

In times of increasing environmental and industrial standards for technologies and innovation toward safer substances closely connected to subsequent legal surveillance in most European Union (EU) countries, it may strike us as strange to communicate that we are confronted with a group of "new" problematical substances in the environment.

Long forgotten are the impressions of great environmental disasters, fish dying in running waters, the disappearance of predatory mammals and birds—scenarios such as these belong to bygone days. For quite some time now, in many EU countries water condition maps indicate a mainstream trend: the status of water quality in Europe seems to be improving (Nixon et al. 2003)—although one should bear in mind that these statements do not refer to the biological status of water quality as required by the newly introduced EU Water Framework Directive (European Parliament and the Council of the European Union 2000).

Today's problematical pollutants are not characterized by high environmental concentrations but occur in the ultratrace range. In many cases even modern analytical techniques are not well enough developed to detect those substances in the subnanogram per liter range. Daughton and Ternes (1999) coined the term "lingering environmental toxicants," identifying personal care products, pharmaceuticals, and endocrine disruptors as integral parts of this substance group. Regarding their common distribution in the environment, optimized biological activity, and effectiveness at comparatively low concentrations, they obviously show some similarities.

However, one distinguishing mark of hormonally active agents is their nonuniform chemical characterization. Therefore, the class of substances possessing hormonal activities incorporates diverse groups such as man-made and natural chemicals (e.g., pesticides, plastisizers, alkylphenol ethoxylates as industrial surfactants, organotin compounds, polychlorinated biphenyls, phytoestrogens, natural hormones) and pharmaceuticals (e.g., ethinylestradiol, methyltestosterone, trenbolone). There is a wide range of biophysical properties, from persistent to rapidly degraded, lipophilic to hydrophilic, and nontoxic to very toxic, that can be attributed to endocrine modulators. Even from a mechanistic point of view, one cannot define a unique mode of action. Although some of the substances take effect by binding but not activating the receptor (antagonistic effect), others may mimic biological activity by attaching to the receptor to produce hormonelike action to an inflationary extent (agonistic effect). Furthermore, endocrine toxicants may act indirectly in a non-receptor–mediated manner when binding to transport proteins (e.g., corticosteroid-binding globulin, steroid hormone–binding globulin, thyroid hormone–binding globulin, growth hormone–binding protein) or by blocking, increasing, and decreasing enzyme activities or other metabolic pathways to affect the synthesis of endogenously produced hormones.

There are a number of less well-known endocrine tissues and hormones other than those producing sexual steroid hormones (e.g., those affecting the hypothalamus and pituitary gland, thyroid and parathyroid glands, adrenal glands, and endocrine pancreas). In any case, a particularly large number of publications on the impairment of reproduction, sexual differentiation, and development caused by hormonally active substances (Colborn et al. 1993; Duft et al. 2003; Jobling et al. 2004) drove research toward a strong focus on sex steroids produced by the gonads.

Endocrine disruption is a global phenomenon. Across the world, endocrine-related effects have been reported in wildlife causing

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reproductive failure and in some cases population declines (Candia Carnevali et al. 2001; Guillette et al. 1994; Kloas 2002; Lavado et al. 2004; Oehlmann et al. 1996). These effects range from feminization of males and virilization of females to suppressed thyroid and immune functions and altered development.

Nevertheless, the greatest attention to endocrine disruption has been paid to estrogenic effects, although a clear cause–effect relationship has been established for the androgenic activities of organotin compounds in mollusks (Bettin et al. 1996; Horiguchi et al. 1997; Oehlmann and Schulte-Oehlmann 2003). In prosobranch snails, tributyltin and triphenyltin compounds interfere with key enzymes of the sexual steroid metabolism. Moreover, these compounds are known also to affect comparable or the same molecular targets in other taxa, including humans (Doering et al. 2002; Heidrich et al. 2001). Therefore, it was self-evident that research within the framework of the EU project COMPRENDO (Comparative Research on Endocrine Disrupters—Phylogenetic Approach and Common Principles Focussing on Androgenic/Antiandrogenic Compounds) would offer the unique opportunity to study a real wildlife–human connection when zooming in on effects mediated by androgen- and antiandrogen-mimicking compounds.

COMPRENDO is one of the four projects that form the core of the CREDO (Coordinating European Environmental and Human Health Research into Endocrine Disruption) cluster and is funded by the European Commission's Fifth Framework Programme for Research, Technological Development and Demonstration activities in the European Community. The overall goal of COMPRENDO is to improve the understanding of the effects of endocrine-disrupting chemicals (EDCs) on aquatic wildlife and humans to improve environmental quality standards and public health in Europe. To this end several key objectives have been identified.

Characterization of Human and Environmental Exposure to Androgenic and Antiandrogenic Compounds

The special issue of EDCs and their potential to cause serious health problems in wildlife species make it questionable whether drinking water and food are still without impact on normal development and sexual differentiation and also whether they allow a normal reproduction and the aging of individuals without avoidable health effects.

A major concern for human health is the suspected intake of EDCs via the diet. To give consideration to the latter in COMPRENDO, human exposure to androgenic and antiandrogenic compounds (AACs) is assessed in all participating European partner countries via food basket analyses (identification of background concentrations in human food). In parallel, an exemplary AAC residue determination in human samples (blood, urine, placenta) in European areas with higher and lower environmental and industrial standards is accomplished. Environmental samples (sediment and biota) are additionally taken and chemically analyzed in these regions. This is to assess the exposure of wildlife populations to AACs as well, and to characterize potential effects in highly contaminated and reference areas.

Impacts of AACs at Environmentally Relevant Concentrations on Human-Relevant Models and Aquatic Species

Presently, the organisms that are used to assess effects of chemicals and in turn infer potential health and ecosystem-level effects are very limited. As new problems arise, we need to be mindful of what chemical tests are the most appropriate and fit for purposes of environment protection rather than necessarily being constrained by existing test systems.

This is why COMPRENDO aims to identify new test species and toxicological end points to help safeguard the protection of organisms living in aquatic ecosystems. To do this a wide range of organisms from invertebrates (echinoderms, crustaceans, mollusks) to vertebrates (fish, amphibians) and human models (human cell lines, tissues, rodents) are under investigation to develop an understanding of the commonalities and differences in responses to AACs across the animal kingdom.

Initially, the application of positive controls for AACs known to cause direct receptor-mediated or indirect functional effects [e.g., methyltestosterone and mibolerone as androgen receptor agonists; letrozole as steroidal aromatase inhibitor; cyproterone acetate, flutamide, and prochloraz as androgen receptor antagonists; 4,7-dimethyl-4-azacholestan-3-one (MK 386) and finasteride as reductase type 1 and 2 inhibitors] provides a basis for the assessment of the potential vulnerability of representative aquatic wildlife groups and humans.

Second, test species are exposed to several compounds [organotins, e.g., mono-, di-, tributyltin and triphenyltin compounds, and pesticides such as fenarimol, vinclozolin, linuron, diuron, *p,p′*-DDE (dichlorodiphenyldichloroethylene)] at environmentally relevant concentrations to identify sensitive and easily measurable end points (genetic, biochemical, physiological, histological, morphological) and critical stages of exposure (via early-life stage and life-cycle tests). These monosubstance tests are linked to sediment exposure studies (sediments originating from environmental sampling) representing complex environmental mixtures. By means of comparison, the results are used to determine whether pure substances or mixtures adversely affect communities in ecosystems.

Establishment of Laboratory Cultures for Suitable Aquatic Invertebrates and Their Baseline Endocrinology

Most of the compounds suspected as endocrine disruptors end up in aquatic ecosystems by sewage effluents. Aquatic organisms are almost continually exposed to these chemicals and, as a matter of plausibility, therefore provide sentinel organisms for detecting adverse biological effects. Determining whether a chemical, especially at sublethal concentrations, can affect endocrine-mediated functions in wildlife populations is difficult and may require a prolonged and profound understanding of the animal's life history, morphology, and the influence of the local environmental conditions (Tagatz 1968).

COMPRENDO aims to support the future research on EDCs with invertebrates. Although it is unquestionable that invertebrates are in principle sensitive to EDCs, their use as standard biotest organisms has been impeded by the lack of laboratory cultures and detailed insights into invertebrate endocrinology. Therefore, the establishment of long-term cultures of selected species of mollusks (*Marisa cornuarietis*, *Potamopyrgus antipodarum*) and crustaceans (*Hyalella azteca*, *Arcatia tonsa*) will be used for studies on endocrine disruption to potentially provide new standard test organisms on the international scale, such as tests mandated by the Organisation for Economic Cooperation and Development (2006) *Inter alia* robustness, synchronicity of development, number of generations per year, ease of handling, diet preferences and the possibilities to achieve a standardization of the diet, cost of culture, and sensitivity of species in the exposures have been important evaluation criteria for checking their suitability for the establishment of laboratory cultures.

The invertebrate reproductive cycle can be highly complex (Sastry 1968, 1970) and is controlled by many environmental stimuli, including light intensity, temperature, desiccation, and diet (Ansell and Trevallion 1967; Copeland and Bechtel 1974; Largen 1967; Tessier et al. 1983; Young 1978). This is why laboratory cultures will finally be optimized in terms of favorable culture conditions (temperate and light/dark regimen, water chemistry, standardized food, animal densities in tanks) and a characterization of the species-specific biology under the chosen culture conditions [reproductive phase(s), time and length of spawning, duration of different development phases, time until puberty, length of sexually active life phase].

Species-Specific Critical End Points, Including a Molecular Screen for Genomic Effects of AACs

On the level of sexual development, reproduction, and differentiation, endocrine modulators may produce a variety of deviations compared with normogenesis and unaffected progeny. Therefore, test species will be checked for pathomorphoses and histopathological aberrations of target organs (e.g., sexual glands, gonads, midgut gland) during exposure. Tracing of cell cycle kinetics, apoptosis, protein content, and mitochondrial respiration provides additional information on disturbances of cytologic processes.

Sex steroid concentrations (androstenedione, testosterone, dihydrotestosterone, 11-ketotestosterone, estrone, and estradiol) are checked in all species under investigation during an unspoiled annual reproductive cycle to be linked with comparative data generated during the exposure's run. The same approach is applied for the determination of phase I and II metabolism. In this regard, the activities of cytochrome P450 aromatase and 3-, 11-, and 17β-hydroxysteroid dehydrogenase are analyzed for phase I. Because interferences with hormone phase II metabolism will alter hormone excretion rates and may lead to hormonal imbalance in exposed organisms, the activities of several enzymes, such as sulfotransferase, acyl-coenzyme A acyltransferase, and sulfotransferases, are analyzed in human cell lines and tissue (placenta, prostate, liver), digestive gland, liver, and gonad of vertebrates and invertebrates in parallel.

In the development of new tests for AACs, innovative approaches are being employed, including the development of a multispecies gene array. Here, a suite of key genes that control sexual function is being isolated and applied to a matrix, and the array subsequently used to screen for the effects of AACs on target gene expression. It is envisioned that this approach will offer new perspectives in the application of modern molecular techniques in our understanding of endocrine disruption.

Identification of Common Principles of an AAC Action across Taxa

The COMPRENDO work program reflects a holistic attempt that will have relevance far beyond the chosen group of compounds. Environmental and human health aspects are simultaneously addressed by using an interdisciplinary and comparative phylogenetic approach. Studies of this nature require a comprehensive understanding of the endocrinology of sentinel organisms and where there are fundamental gaps of knowledge in baseline endocrinology (notably in invertebrates).

A common and distinguishing mark of the endocrine system of all animal phyla consists of a widely distributed system of glands, tissues, and diffuse groups of widespread cells that synthesize and release chemical messengers into the circulatory system. The latter implement their regulatory effects at various target sites within the body.

Doubtless the regulation of many parts of biological processes has been widely conserved, although individual components of the endocrine system have passed through profound evolutionary processes. Consequently, remarkable divergences resulting in distinct differences in the endocrine systems of the diverse taxa can be expected (de Fur et al. 1999), but on the other hand, we have reason to suppose that in the animal kingdom many similarities and analogues of metabolic pathways also exist.

It is likely that genes, proteins, or receptors differ in structural configuration across taxa, and even if the differences are very small, they may constitute the basis for different effects and reactions in test species when exposed to the same substances and concentrations. For example, conventional radioreceptor assays have provided evidence for androgen- and estrogen-specific binding sides in mollusks, crustaceans, and echinoderms (Oehlmann et al. 2006). Nevertheless, their binding affinity to direct-acting AACs varies enormously compared with those of vertebrates. Although parts of vertebrate-type sex steroid metabolism can be identified in the different invertebrate test species, the conversion rates from androgens to estrogens might be identified to be much lower compared with those of vertebrates. Different activity patterns of the enzymes involved (occurrence, function, biosynthesis) or other interspecies differences with respect to the metabolic pathways (e.g., conjugating activities) may turn out to be responsible for this.

However, these and other findings make it worthwhile to clone and sequence the androgen and estrogen receptors in the different species under investigation and to focus on deciphering their endocrine systems. Finally, this could pave the way toward an extrapolation of data derived from animal experiments to human health based on factual knowledge and improve our knowledge on the extent and nature of endocrine disruption by AACs with a critical evaluation of their role as disruptors of physiological functions in wildlife and human populations (risk assessment).

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