

## 1. EXECUTIVE SUMMARY

Contract no	<i>EVK1-CT-2002-00129</i>	Reporting period	1 October 2004 to 31 March 2005
Title	COMPRENDO: Comparative Research on Endocrine Disrupters – Phylogenetic Approach and Common Principles focussing on Androgenic/Antiandrogenic Compounds		
<b>Objectives</b> <p>The overall goal of COMPRENDO is to improve the understanding of the effects of endocrine active compounds on aquatic wildlife and humans, focussing on androgenic and antiandrogenic compounds (AACs). This will help to improve environmental quality standards and also the public health in Europe. To this end the key objectives are to:</p> <ul style="list-style-type: none"><li>• Characterise the human and environmental exposure to AACs</li><li>• Determine the impacts of environmentally relevant doses/concentrations of AACs on a wide range of human-relevant models and aquatic species</li><li>• Develop new biological effect measures and species-specific critical endpoints, including a molecular screen for genomic effects of AACs</li><li>• Identify common principles of AAC action in different species and to develop new animal models for extrapolation to human health</li><li>• Develop laboratory cultures for suitable aquatic invertebrates and to establish their baseline endocrinology</li><li>• Characterise the risk originating from AACs in humans and wildlife.</li></ul>			
<b>Scientific achievements</b> <p>During the reporting periods the following scientific achievements were attained:</p> <ul style="list-style-type: none"><li>• Analytical measurement of p,p'-DDE and DDT in human food samples have been completed for the first two sampling campaigns. High contaminations of these organochlorine pesticides have been quantified in fatty foods (except milk), eggs, meat and fish. In case of the latter nearly all samples contained between 2-20 µg DDE+DDT/kg, irrespective of the European country the food samples originated from. Furthermore some general trends have been derived for the organochlorine analyses: DDE levels were much higher in foodstuffs sampled during the second campaign (summer time) and average concentration range was between 0.8 to 3 µg /kg. The different food categories, fish and fatty oils turned out to contain the highest DDE concentrations, followed by cheese, eggs, meat and shellfish. Very low levels of both contaminants were detected in vegetables, fruits, beer and wine.</li><li>• Androgen and anti-androgen laboratory exposure experiments with human cell tissue, cell lines, rats, amphibians, fish, echinoderms, crustaceans, and molluscs have been completed. The results of these experiments demonstrated that all test systems were affected by the test compounds applied on different levels of examination. Androgen mimicking substances, such as organotins and some pesticides, e.g. resulted in the induction of virilization, a reduced reproductive performance, an accelerated sexual maturity, reduced larval development, reduced skeletal density, misaligned sex ratios and various effects on reproductive organs under histopathological investigation. Feminization effects, reduced egg diameters, the lack of spermatids and spermatophores have been observed under anti-androgen exposure.</li><li>• Receptor binding studies for ER/AR revealed the existence of estrogen and androgen specific binding sides in <i>Paracentrotus lividus</i>, <i>Antedon mediterranea</i>, and <i>Marisa cornuarietis</i> whereas in <i>Hyalella azteca</i> only an androgenic binding side (not interfering with ecdysteroids) was</li></ul>			

detected. The following nuclear receptors have now been detected: in *Marisa* ER-like, ERR-like, E75-like, RAR-like and RXR-like, in *Paracentrotus*; ERR-like, RAR-like and RXR-like, in *Hyalella*, ERR-like. The full sequence for *Marisa* ER-like has been attained, and the *Marisa* ERR-like sequence is now almost complete.

- Cytotoxicity assessments using the MTT and AlamarBlue assays resulted in low dose effects with stimulation effects compared to the controls. Cytotoxic effects, evaluated as inhibition in the assays, were only observed at very high concentrations for some of the androgenic compounds.
- COMPRENDO has also made efforts to elucidate the similarities and differences in the steroid metabolism of test species. It is important to note that as well as the striking similarities between vertebrate and invertebrate responses to AACs, there are also differences in the patterns of responses to the different chemicals between the different organisms. It is obvious, for example that the effects of AACs on steroidogenesis differ between the fish and the mollusc and between the mollusc and the crustacean. By studying the steroidogenesis in these organisms it becomes obvious that these differences in responses to AACs are probably due to differences in the steroidogenic pathways. Notwithstanding this, these large differences in steroidogenic pathways may also be observed by comparing fish and amphibians (in other words, two vertebrates) as well as vertebrate and invertebrate.
- Two of the organisms being used within COMPRENDO are being considered by OECD as suitable test organisms for assessing endocrine disruption:

The draft OECD method on calanoid copepods (*Acartia tonsa*) has been prepared . Pre-validation work is currently running. Based on the results of this, the methods will be refined and a ring-test will then be run later this year.

The OECD *Ad hoc* Expert group on Invertebrate Testing has agreed that the prosobranch snail *Potamopyrgus antipodarum* is the preferred molluscan species for the development of a test method. As a first step in the generation of a draft protocol, a standard operating procedure for the culturing of *P. antipodarum* and for the 56 d-reproduction test has been prepared and the new version will be available in summer 2005.

### **Socio-economic relevance and policy implications**

The project contributes to the identification of potential threats to quality of life, health and safety in Europe. The special issue of endocrine active chemicals (EACs) and their potential to cause serious health problems in humans and wildlife species at extremely low doses makes it questionable whether drinking water and food are still without impact on normal development, sexual differentiation and also whether they allow a normal reproduction and the aging of individuals without avoidable health effects. Costs of future medical treatments of EAC-related diseases and disorders are likely to be very high if these compounds interfere with human health aspects. An identification of chemicals as EACs would make it necessary to take restrictive measures to avoid further harm to humans and wildlife.

Two new test species are proposed to the OECD, regarding the development of guidelines for chemical testing to provide new toxicological endpoints to ensure the protection of organisms within the aquatic ecosystems but also to improve the protection of human health. Many of the current results are still preliminary, especially in view of baseline endocrinology in invertebrates but will provide important contributions to the exploration of functionality of the steroidogenesis in the animal kingdom. Nevertheless, the existing results indicate that new test species and new toxicological endpoints are identified to support the international search for new EAC-specific standard tests and to facilitate the current efforts for new approaches regarding the Environmental and Human Risk Assessment for EACs.

### **Conclusions**

The exposure of humans and wildlife species to selected AACs can now be assessed in different matrices. Most of the results are available for the effects of these compounds in

aquatic invertebrates and vertebrates as well as human-relevant test systems on different levels of biological organisation (from molecules to organisms). For the remaining life of the project it is anticipated that all results of exposures, morphological, histological, chemical and physiological measurements will be appropriately evaluated to position consolidated findings in the overall context of endocrine disruption in human and wildlife. The first evaluations of the data material generated within the project as well as literature inquiries on substance information available are already on the way.

**Keywords:** endocrine disrupters, human health, environmental risk, exposure, effects