

1. EXECUTIVE SUMMARY

Contract no	<i>EVK1-CT-2002-00129</i>	Reporting period	1 October 2002 to 31 March 2006
Title	COMPRENDO: Comparative Research on Endocrine Disrupters – Phylogenetic Approach and Common Principles focussing on Androgenic/Antiandrogenic Compounds		
Objectives <p>The overall goal of COMPRENDO was to improve the understanding of the effects of endocrine active compounds on aquatic wildlife and humans, focussing on androgenic and antiandrogenic compounds (AACs). This was meant to help to improve environmental quality standards and also the public health in Europe. To this end the key objectives have been to:</p> <ul style="list-style-type: none">• Characterise the human and environmental exposure to AACs• Determine the impacts of environmentally relevant doses/concentrations of AACs on a wide range of human-relevant models and aquatic species• Develop new biological effect measures and species-specific critical endpoints, including a molecular screen for genomic effects of AACs• Identify common principles of AAC action in different species and to develop new animal models for extrapolation to human health• Develop laboratory cultures for suitable aquatic invertebrates and to establish their baseline endocrinology• Characterise the risk originating from AACs in humans and wildlife.			
Scientific achievements <p>During the reporting periods the following scientific achievements were attained:</p> <ul style="list-style-type: none">• The investigations on food contamination (cereals, potatoes, eggs, apple, butter, oil, meat, milk, wine, beer, orange juice, cheese, fish and sea food) with pesticides, organochlorines and organotin compounds of the participating European countries (Greece, Spain, Italy, France, United Kingdom, the Netherlands, Denmark, Sweden, Poland, Germany) resulted in the following achievements: 35.6% (390 samples) of all samples contained AAC residues, but only in 1.1% of the latter the measured values exceeded the MRL (maximum residue limit) guideline values according to EU directives. Considering the national eating habits and consumerism in none of these cases an exceedance of ADI (Acceptable Daily Intake) values was observed. However, the latter refer to single substance intake whereas aliment contain a complex mixture of chemical residues wherefore these data do not allow to finally decide on which impacts even low AAC contaminations may have on the individual health of consumers. Furthermore current safety regulation for consumer health protection does not consider endocrine effects.• Human blood sera of German and Polish donors have been analysed regarding steroid levels of testosterone, 17-β-estradiol, androstendione and dihydroepiandrosterone. Donors have been divided in occupationally exposed subgroups of farmers, dockers, frequent fish eaters and Polish and German non-occupationally exposed “control” groups. No significant differences between subgroups were found when comparing testosterone and dihydroepiandrosterone levels. However, the mean value of 17-β-estradiol in the German “control” group was significantly lower compared to all Polish groups. Androstendione measurements revealed lower values for German “controls” compared to Polish “controls”, dockers and fish eaters. Furthermore Polish			

farmers had lower androstendione values compared with Polish dockers. In summary, there only minor differences between mean values of the different sub-groups. No significant correlation between sex steroid concentration and the chemical residues in blood of the donors was found.

- Cytotoxicity assessments using the MTT and AlamarBlue assays in human cell lines 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.
- Anabolic effects manifesting in disproportionate cell growth of ER/AR-positive (MCF7, LNCap-FGC) human breast and prostate cancer cell lines when exposed to low doses of AACs have been observed. If present in vivo it cannot be excluded that this may also affect tissue homeostasis.
- Androgen and anti-androgen laboratory exposure experiments with human cell tissue, cell lines, rats, amphibians, fish, echinoderms, crustaceans, and molluscs have been performed. The results of these experiments demonstrated that all test systems were affected by the test compounds (TBT, TPT, linuron, diuron, fenarimol, vinclozolin, p,p'-DDE) either on different species-specific or comparable 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, changes in sex steroid titres and enzyme activities and various effects on reproductive organs under histopathological investigation. Feminisation effects, reduced egg diameters, the lack of spermatids and spermatophores have been observed under anti-androgen exposure.
The observed effect concentrations were in the range of environmental relevant concentrations and partially resulted in new PNEC (Predicted No Effect concentration)-values for risk assessment

- For six of seven chemicals under investigation a toxicological assessment was possible. For five substances the calculations resulted in risk assessment factors >1 for freshwater ecosystems and for three substances in factors >1 for marine ecosystems, indicating a risk for the environment. Effect concentrations derived from chronic COMPRENDO exposure experiments (including life-cycle- and 2-generation-tests) gave considerably lower values than provided by literature for the chemicals fenarimol, triphenyltin, linuron and p,p'-DDE.
These low effect concentrations have been the determining factors in view of risk assessment and finally led to calculations of lower PNECs (Predicted No Effect Concentrations) and higher risk factors. However the COMPRENDO analysis did not inevitably produce higher risk factors. COMPRENDO experiments e.g. allowed for the consideration of additional trophic levels. The latter added up to lower assessment factors and consequently a lower risk factor for the presence of vinclozolin in freshwater ecosystems.

- Two of the organisms being used within COMPRENDO are considered by the OECD as suitable test organisms for the OECD guideline programme on testing of chemicals (including the assessment of endocrine disruption):

The draft OECD method on calanoid copepods (*Acartia tonsa*) has been prepared. A ring-test for validation is currently running.

Furthermore the OECD *Ad hoc* Expert group on Invertebrate Testing has agreed upon the prosobranch snail *Potamopyrgus antipodarum* as preferred molluscan species for the development of a test guideline. As a first step toward validation and generation of a draft guideline, a standard operating procedure for the culturing of *P. antipodarum* and for the 56 d-reproduction test (water and sediment) has been prepared. The document is available on request to the co-ordinator.

- 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 differences in steroidogenic pathways may also be observed by comparing fish and amphibians (in other words, two vertebrates) as well as vertebrate and invertebrate.

- Receptor binding studies for ER/AR revealed the existence of estrogen and androgen specific binding sites in *Paracentrotus lividus*, *Antedon mediterranea*, and *Marisa cornuarietis* whereas in *Hyalella azteca* only an androgenic binding site (not interfering with ecdysteroids) was 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. The function of these receptors are yet not distinctly clarified.
- The research on molecular markers yielded to the development of a macro-array prototype composed of the genes of diverse fish species. A first validation run with the aromatase inhibitor fenarimol confirmed the operating mode of the array. The molecular responses complied with phenotypic observations made under fenarimol exposure, wherefore the application of the array for the assessment of AAC effects is most promising.

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.

The consortium was able to propose two new test species to the OECD, regarding the development of guidelines for chemical testing. This was to provide new toxicological endpoints to ensure the protection of organisms within the aquatic ecosystems but also to improve the protection of human health.

There are still large gaps in knowledge of baseline endocrinology, especially in view of invertebrates. COMPRENDO provided important contributions to the exploration of functionality of the steroidogenesis in the animal kingdom. Therefore our results will facilitate the current efforts for new approaches toward the improvement of Environmental and Human Risk Assessment for EACs – on national and international scale.

In summary it can be stated that the observed effects under chemical exposure in vertebrates and invertebrates nevertheless suppose the existence of highly conservative configured pathways in the endocrine system throughout the animal kingdom. Especially in view of the pre-arrangements toward a EU-wide ban of vertebrate animal testing in cosmetics industry in 2009 and a general ban in 2013, invertebrate test species may present a reasonable alternative.

Conclusions

The exposure of humans and wildlife species to selected AACs has been assessed for different matrices. The results on the outcome of exposure experiments are available for different levels of biological organisation (from molecules to organisms) in aquatic invertebrates, vertebrates and human-relevant test systems. An evaluation of the data considering new inputs for ongoing risk assessment procedures has been conducted.

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