

MULTI-ORGANIC RISK ASSESSMENT OF SELECTED ENDOCRINE DISRUPTERS (EURISKED)

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Contract n°	EVK1-CT-2002-00128	Reporting period: 3	1.10.2004-30.9.2005
Title	Multi-organic Risk Assessment of selected Endocrine Disrupters		
<p>Ultimate goal of the EURISKED project was to determine multi-organic effects of a variety of endocrine disrupters (EDs); these are: OMC, Nonylphenol, 4-MBC, Bisphenol A, Dibutylphthalate, Benzophenone-2, Procymidon, Linuron, Resveratrol, 8-prenylnaringenin, genistein, estradiol-benzoate, androstadiol. The plant derived substances and the plasticisers have been shown to be uterotrophic, i.e. to have estrogenic effect in the uterus, while the 2 pesticides prevented androgen stimulated prostate growth. Since these steroids, but also thyroid hormones and glucocorticoids have profound effects in many other organs outside the reproductive tract it was a goal of this project to study these effect outside the reproductive tract. As a control, targets within the reproductive tract were incorporated in the study design as well.</p> <p>Molecular and cell biological experiments as well as research in animals and in the human indicate that ED's with estrogenic actions exist, which are present in either cosmetics (such as UV-absorbers and stabilisers) or pesticides / fungicides. Little research has been done as to whether these substances interact with other steroid receptors or act in non-reproductive organs like the neuroendocrine brain, the cardiovascular, skeletal or urogenital system during development and adult life. Hence, risk assessment for organs known to be estrogen-, androgen-, progesterin-, glucocorticoid- or thyroid hormone-receptive following exposure to the above mentioned endocrine disrupters could not be made on the basis of the data available at the time of the beginning of the project. To study such effects with basic experimental and clinical tools represents the fundamental objective of this RTD project.</p> <p>Many ED's have been defined as ligands to a variety of steroid hormone receptors, their effects being studied in a multitude of cell lines. Many diseases have been related to pre- or postnatal exposure to ED's and long-lasting effects need to be studied. In-vivo experiments in rats and mice will be performed, in which dams and newborn pups received substances known to be either estrogenic or antiandrogenic or to have antithyroid hormonal effects, namely 2 UV-absorbers used in the production of sunscreens, 1 stabiliser used in cosmetics, 1 fungicide used in fruit plantations, 1 pesticide and 1 synthetic flavone with antithyroid effects. In addition, adult gonadectomised or adrenalectomised rats and gene-targeted mice (steroid receptor knock-out mice) were also fed with the substances Estrogenic, androgenic, progestational, glucocorticoid, and thyroidal effects will be studied with genomic and proteomic tools in the brain and in the cardiovascular, skeletal and uro-genital systems. Experiments with steroid-receptor knock-out mice proved ultimately as to whether the substances of interest have steroid hormone receptor mediated activities in the intact organism. 5 of the 18 substances with known ED activity on the OECD list released recently were studied and those displaying multi-steroidal effects were incorporated into the main study. Clinical trials were performed with commercially available UV screens. The effects of phytoestrogens on the cardiovascular system (including the lipid profiles) and the bone were also determined. Transdermal resorption of UV-absorbers and their temporal presence in the body were studied in rats and human subjects</p> <p>Scientific achievements: By means of histology, including immunocytochemistry it was shown that all uterine parts (endometrium, myometrium) were stimulated by the phytoestrogens, whereas 4-</p>			

MBC and OMC had very mild uterotrophic effects. Linuron and Procymidone were ineffective. 8-Prenylaringenin, a hop derived flavonoid caused formation of polypoid structures in the endometrium, an effect which has never been published before. In the mammary gland all phytoestrogens clearly stimulated the formation of the nuclear proliferation marker Proliferating Cell Nuclear Antigen (PCNA) and of the progesterone receptors, both are very typical estrogenic effects. Under higher doses, the phytoestrogens stimulated also development of mammary gland ducts and milk production. If occurring also in the human, this would endanger the uterus and the mammary gland to develop malignant tumours. In the thyroid most test substances inhibited either thyroidperoxidase, which may endanger the organism to become hypothyroid, or thyroid hormone deiodinases. Some of the tested UV filters proved to be estrogenic, an undesired effect could occur if the substances are resorbed transcutaneously. These investigations were performed in the 3rd year; the measurement performed so far did not result in detectable serum levels of the UV screens when commercially available products were tested in female and male patients. The present method may not be sufficiently sensitive, therefore the remaining serum samples of all test substances are being analysed in cooperation with partner 3, who has developed a highly sensitive estrogen reporter cell systems. The 2 pesticides tested inhibited testosterone induced growth of prostates and seminal vesicles. This anti-androgenic effect may have adverse effects in pubertal boys not only in the development of prostate or seminal vesicles but also in bone maturation. These results will enable the consortium to focus in the future in some more details in the various mouse and rat models and this will be of major importance to estimate the putative danger for the human.

Main deliverables:

The experiments allow risk assessment in developing and adult animals not only in reproductive but also in other steroid-receptor organs (Milestone 1). The molecular mechanisms behind these actions were elaborated in the brain, the cardiovascular system and the bone (Milestone 2). Risk assessment for the non-reproductive organs was also possible in the human (Milestone 2)

The results obtained testing the 11 studied endocrine disrupters allow differentiation between substances with estrogenic, anti-androgenic, thyroid affecting and undefinable effects. The estrogenic substances were “given in consecutive order of uterotrophic strength” Benophenone-2 > 8-Prenylaringenin > 4MBC > OMC > Bisphenol A > Dibutylphthalate. The latter three compounds were almost devoid of uterotrophic effects. Resveratrol, Procymidone and Linuron proved to be ineffective in the uterus. Clear anti-androgenic effects were observed for Procymidone and Linuron. The thyroid function was affected by 4MBC > OMC > 8-Prenylaringenin > Genistein. Molecular mechanisms involved in altering thyroid hormone levels involve not yet clearly established hypothalamic/pituitary mechanisms, inhibition of thyroid peroxidase and inhibition of deiodinases in various organs. The effects of some substances like 4-MBC at a high dose could not be explained on the basis of interaction with steroid receptors.

Socio-economic relevance and policy implications:

While estrogenic and anti-androgenic effects of some of the studied endocrine disrupters were intensively investigated also internationally, the effects in the thyroid gland and in the adrenal open totally new perspectives, which clearly deserve further investigation. Here, food additives on the market to replace hormone replacement therapy (soy, red clover, genistein) or used for cosmetic purposes like bust enhancement (8-prenylaringenin) or as anti-ageing compound (Resveratrol) need further attention. Since genistein and 8-prenylaringenin do not only interfere with thyroid hormones but had strong estrogenic effects, their safety concerning uterus and mammary gland should

be thoroughly investigated. It should thus be a political goal to make European food safer, to sponsor research addressing the safety of isoflavone containing foods, particularly in view of the potency of their estrogenic effects in the mammary gland and in the uterus, which may endanger these organs for the development of cancers.

Concerning the anti-androgenic effects of Linuron and Procymidone it was not in the scope of this project to compare effects obtained in the laboratory with those that might occur in heavily exposed humans. However these pesticides are widely used in agriculture and therefore rigid control of food, particularly maybe foodstuff, is adviseable.

The plasticizers had very moderate effects in all test systems used by the consortium and therefore they appear not to be of major concern when consumed. However in concert with other EDC they may add to endocrine disrupting effects.

Conclusions:

With cell biological, molecular, animal experimental and clinical studies the putative adverse effects of a number of tested EDCs in the thyroid system, the mammary gland and the uterus were established. The putatively dangerous effects warrant further exploration and political activities

Dissemination of results:

These results were disseminated primarily in scientific media (congresses, progress report of EU funded projects and scientific publications). Most consortium members also had close contact to local news media (TV, Newspapers, Public journals).

Keywords: Endocrine disrupters, risk assessment, multi-organic

Publications (cumulative list)

Peer Reviewed Articles:

Resulting directly from EURISKED (acknowledging contract)

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