

## Work Package 7: Endocrine Disruptors

Endocrine disruption due to chemical contamination has been a growing concern in the field of food safety for several years. The presence in food and/or the environment of chemicals that can interfere with the endocrine system and compromise homeostatic equilibrium may represent a real risk to human health. The BioCop project will specifically take into account the risks of exposure to such chemicals for highly susceptible populations: the risks of endocrine disruption to the foetus and young children, during critical stages of development are of particular concern.

The number of chemicals identified as endocrine disruptors is continuously increasing. They include natural substances (endogenous hormones, phytoestrogens etc.) and artificial compounds (pesticides, phthalates, alkylphenols, persistent organic pollutants). Some of these classes of chemicals are considered in other BioCop work packages (steroid hormones in WP8, organochlorine pesticides in WP6). WP7 will therefore focus on the phytoestrogen family.

The main objective of WP7 is to develop efficient confirmatory methods, based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), for identification and quantification of at least 4 target analytes: daidzein, enterolactone, coumestrol and resveratrol (Figure 1) which are representative of the main classes of phytoestrogens (isoflavones, lignans, coumestans and natural stilbens) found in milk, cereal and baby food sample.

The aim will be the characterisation of a panel of food products in terms of phytoestrogen content, before transferring the extracts to WP1. WP1 will then identify specific reporter genes that are activated or inhibited due to the action of phytoestrogens in specific cell lines.

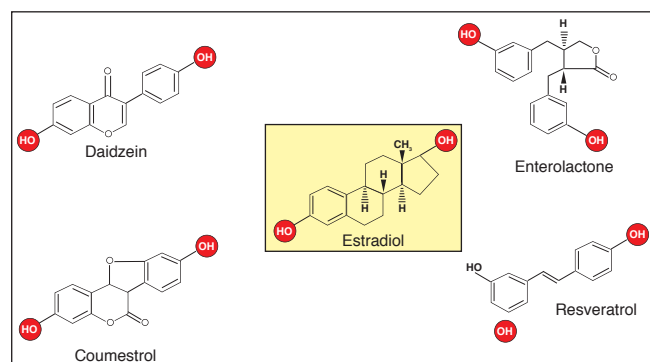


Figure 1: Target analytes (daidzein, enterolactone, coumestrol and resveratrol)

The main expected result of this study is to determine a selection of genomic biomarkers whose activation is indicative of exposure to phytoestrogens. The ultimate aim is then to use these genes in creating a DNA chip system for end-users and laboratories in charge of contamination control in food safety. The data generated regarding the molecular mechanisms of action of these substances will also be useful for more fundamental biological and toxicological purposes.

### Importance of the analytes selected

Although some beneficial effects have been reported for phytoestrogens, their adverse effects on human health have also been highlighted. These negative aspects are linked to their very high structural similarity with natural active oestrogens and their capability to bind with the oestrogen receptor. Some *in vitro* and *in vivo* studies have also shown an ability to induce proliferation of specific mammalian cells, with potential carcinogenic consequences. The consumption of phytoestrogens by healthy adults may not be harmful, however, exposure occurring during development (in utero and in young children) may be problematic. Soymilk derivatives, recently introduced in baby-food, should, therefore, be checked for potential adverse effects. Another possible source of phytoestrogens is cows' milk as a consequence of using vegetable/soya flour in animal feed.

### Current state of the art

The techniques currently used for analysing phytoestrogens are immunochemical methods and chromatography, coupled to mass spectrometry, which provide a better sensitivity and specificity. Until recently, mass spectrometric methods only concerned a limited number of analytes (mainly isoflavones) and provided detection capabilities not better than the ng/ml (parts per billion) order. Moreover, the analytical procedures did not take into account all potential precursors (glucosides, methoxylated) and/or metabolites present in feed and/or food. The procedures, being complex and expensive, are not suitable for generic detection and characterisation of the potential endocrine disrupting activity of contaminants.

Fast and generic screening methods for endocrine disruptors, especially phytoestrogens, for both food and feed products and for exposure assessment through animal

or human biological fluids are urgently needed, as are efficient in-line or off-line confirmatory techniques based on mass spectrometry, for the regulatory aspect.

## Advancement beyond the state of the art

BioCop is developing fast and efficient methodologies for large scale screening of endocrine disrupting chemicals. Transcriptomic methods using microarray DNA chips will be adapted (WP1) to overcome the limitations of current bioassay screening methods. A repertoire of genes will be established that are specifically regulated in cultured human cells in response to the analytes of interest, i.e. phytoestrogens, organochlorines and trichothecenes. This information will be used to construct a new high-throughput DNA microarray platform tailored to the specific requirements of food residues and contaminant screening. Relative to existing bioassays, this multi-endpoint approach will expand the range of substances that can be detected in a single set up and will improve the specificity of the results. Multi-residue extraction methods will also be developed, reducing the matrix complexity without causing significant loss in analyte composition. The final outcome is to deliver a new technological platform with broad screening capability. These techniques are expected to achieve a higher level of efficiency than ever before, in terms of sensitivity and range of analytes, due to the focus on global endocrine disrupting potency and not on specific compounds.

The first expected practical result will be the possible application of the developed assays for rapid exposure assessment of various food products and biological fluids. Another important challenge will be to provide important information regarding the molecular mechanisms of action of endocrine disruptors. BioCop will allow the development of a rapid and generic screening method for endocrine disruptors based on a transcriptomic approach. With BioCop expertise, new extraction and purification protocols for endocrine disruptors in biological samples will also be developed. Confirmatory methods, based on mass spectrometry, will be optimised to validate data generated from the screening analysis of different matrices. The same range of compounds and concentrations will be covered as those included in the new screening technologies.

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