



**QUALITY OF LIFE AND MANAGEMENT OF  
LIVING RESOURCES**



**ENVIRONMENTAL AGENTS SUSCEPTIBILITY ASSESSMENT  
UTILISING EXISTING AND NOVEL BIOMARKERS AS RAPID  
NON-INVASIVE TESTING METHODS**

**Contract n°QLK4-2002-02286**

**Project Progress summary**

**Reporting period I-XII 2003**

<b>Title of the project</b>		
ENVIRONMENTAL AGENTS SUSCEPTIBILITY ASSESSMENT UTILISING EXISTING AND NOVEL BIOMARKERS AS RAPID NON-INVASIVE TESTING METHODS		
<b>Acronym of the project</b> <b>EASYRING</b>		
<b>Type of contract</b> <b>RTD</b>		<b>Total project cost</b> (in euro)
		2351,056 €
<b>Contract number</b>	<b>Duration</b> (in months)	<b>EU contribution</b> (in euro)
QLK4-2002-02286	36 Months	1890,209 €
<b>Commencement date</b>		<b>Period covered by the progress report</b>
01/01/2003		1 January 2003 – 31 December 2003
<b><u>PROJECT COORDINATOR</u></b>		
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<b>Key words</b> (5 maximum - Please include specific keywords that best describe the project.).		
Endocrine disrupters, Biomarkers, Risk assessment, <i>in vitro</i> , dipstick		
<b>World wide web address</b> (the project's www address )		
WWW.easyring.org		

**List of participants** Provide all partners' details including their legal status in the contract i.e., contractor, assistant contractor (to which contractor?).

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## Objectives:

The major objectives and achievements expected for EASYRING are:

- Rapid, non-invasive test development for the identification of specific biomarkers for endocrine disrupting chemicals in the mucus of aquatic species.
- Novel functional biomarker(s) for the identification of endocrine disrupting chemicals.
- Production and validation of new enzyme linked immunosorbent assays (ELISAs) for the detection of new biomarkers in plasma, mucus and tissues of aquatic species.
- Improvement of *in vitro* test for the screening of endocrine disrupting chemicals.
- Development of analytical methods in GC-MS and LC-MS for selected endocrine disrupting chemicals in environmental water and biota.
- Short/long term and low level exposure effects of selected chemicals and mixture in low vertebrates.
- Short/long term and low level exposure effects of selected chemicals and mixture, through food and water chain, in small mammals.
- QSARs development for the prediction of chemicals able to elicit endocrine disruption.
- QAARs development to extrapolate the responses of the different experimental models.

## Results and Milestones:

- 1) Identification of the fractions of environmental extracts showing the highest endocrine disrupting potentials when tested with the same battery of *in vitro* methods.
- 2) Development of new methods in GC-MS and LC-MS for selected endocrine disrupting chemicals in environmental water and biota (M17).
- 3) Development of assays on gamete maturation and activation (M20).
- 4) Protocol for identification of short/long term and low level exposure effects of selected chemicals and their mixtures in aquatic species (M3).
- 5) Protocol for identification short/long term and low level exposure effects of selected chemicals and their mixtures in small mammal (M4).
- 6) Pilot studies for the establishment of a specific protocol to speed up and simplify detection of candidate biomarkers in carps.
- 7) Initial protocol for immobilisation of antibodies in simple test system (M9).
- 8) Fish community characterisation in the Po river.
- 9) Establishment of retinol binding protein as new biomarker in *Xenopus* (M1)
- 10) Collation of data from the literature on endocrine disrupting activities and formulation of data-base (M25)
- 11) Brochure
- 12) Website

**Benefits and Beneficiaries:**

- Improvement of the standard validated tests for risk assessment (*beneficiaries*: policy makers, scientist, national and regional environmental agencies, environmental quality monitoring agencies and laboratories)
- Development of new non invasive test methods (*beneficiaries*: policy makers, scientist, national and regional environmental agencies, environmental quality monitoring agencies and laboratories)
- Improvement of *in vitro* test systems with alternative screening and testing protocols (*beneficiaries*: scientist, environmental quality monitoring agencies and laboratories)
- Improvement of chemical analytical method and their application (beneficiaries: scientist, environmental quality monitoring agencies and laboratories)
- Improvement of knowledge on Cytotoxic and teratogenic effects of endocrine disrupters/modulators and quantification of exposures in mammals (cause-effects relationships investigation) (*beneficiaries*: policy makers, national and regional environmental and health agencies, scientist).

**Future Actions (if applicable):**

During the second year of the project, activities will be performed following the original timetable foreseen in the technical annex. Even though, due to the reproductive behaviour of carps (bounding to perform *in vivo* exposures at the end of summer to wait for the resting period), new biomarker(s) detection had been delayed, the Consortium set up strategies in order to solve the problem and close the gap. Moreover, during the first intermediate meeting, it was planned to perform a K1 carp caging in two sites located upstream and downstream the Lambro river confluence. This step was not foreseen in the original project, but it is considered a helpful tool to better understand data coming from the in lab exposures to chemicals selected on the base of *in vitro* testing and analytical analyses.