EU Integrated Project - ReProTect - Development of a Novel Approach in Hazard and Risk Assessment for Reproductive Toxicity by a Combination and Application of *In Vitro*, Tissue and Sensor Technologies. (2004 - 2009)

Study goal

The aim of the "ReProTect" project was to integrate existing and newly developed *in vitro* models into a testing strategy that can provide detailed information about the potential hazard of compounds to reproduction.

Background

Reproductive toxicity testing of industrial chemicals under the new EU Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (EU, 2006) will require up to 60% of experimental animals foreseen for the entire testing of existing chemicals (Pedersen *et al.*, 2003) (more updated but highly controversial data are available in literature see EU, 2009).

Furthermore, the 7th amendment of the Cosmetics Directive 76/768/EEC foresees the phasing out of *in vivo* testing of finished cosmetic products (since September 2004) and ingredients or combination of ingredients (since March 2009) (EU, 2003). Moreover, the marketing ban for finished cosmetic products and their ingredients that were tested on animals is in place since March 2009 referring to the all human health effects with the exception of repeated-dose toxicity, reproductive toxicity and toxicokinetics. For these complex systemic toxicological endpoints, the marketing ban entered into force in March 2013. Therefore, there is an urgent need for the availability of alternative replacement methods. It is therefore expected that new alternative test strategies could provide more detailed information about the toxicological mechanisms which is essential to improve the risk assessment of chemical compounds. It may also lead to more efficient drug development and undesired effects may be discovered in earlier stages.

To meet these urgent needs for alternative test methods within the reproductive toxicity area, the EU Integrated Project "ReProTect" (**www.reprotect.eu**) was launched. The project is founded by the European Commission within the EU 6th Framework Programme for Research, Technological Development and Demonstration, assembling 33 different European partners from academia, small medium enterprises, governmental institutions and industries.

Objectives

The overall aim of the "ReProTect" project is to develop new *in vitro* models and to improve existing ones, in order to integrate them into a testing strategy, which aims to provide detailed information on the potential adverse effects of chemical compounds to the mammalian reproductive cycle.

The principal objectives of the project:

- To replace animal experimentation or reduce the number of animals used within reproductive toxicity testing of chemical compounds.
- To improve the characterisation of toxicological mechanisms.
- To improve the risk assessment process of substances.

Description

Detailed information on this project can be found in the official ReProTect project website (**http://www.reprotect.eu/** last accessed: November 2012). The project has been structured into four research areas:

- Germ cells, steroidogenesis and fertilisation
- Embryo Implantation
- Prenatal development
- Crosscutting technologies; to explore the applicability of innovative methods within reproductive toxicity testing.

A series of workshops were held to discuss the current status of *in vitro* and *in silico* methods applicable in each reproductive toxicity topic area after which a development and a prevalidation activity of selected *in vitro* tests was planned.

Outcome

In the "ReProTect" project the reproductive cycle was broken down into its biological components, in order to define and further develop *in vitro* systems able to assess chemical hazards in the areas of: male/female fertility, implantation and prenatal development. Furthermore, innovative methods, such as proteome analysis, quantitative structure-activity relationships (QSARs) and microarray technologies have been included in the area of “Cross-cutting technologies”.

More than 20 different tests or test systems were investigated, reflecting various toxicological endpoints aiming to measure effects on spermatogenesis, folliculogenesis, germ cell maturation and fertilisation, steroidogenesis, the endocrine system, the pre-implantation embryo, placentation, uterus function and embryonic development.

More than 150 peer-reviewed reproductive toxicants have been tested. Scientific information on the presumed mechanism of the various test chemicals has been collected and standard operating procedures (SOPs) for most of the tests have been produced.

Tests or test systems under research within the "ReProTect" project (Hareng *et al.*, 2005):

Fertility:

- Computer Assisted Semen Analysis (CASA)
- Sperm Chromatin Structure Assay (SCSA)
- Neutral Comet Assay in Sperm (ReProComet)
- Leydig Cell-enriched Cultures
- Sertoli Cell Enriched Cultures
- *In Vitro* Fertilisation Assay
- Follicle BioAssay (FBA)
- Granulosa and Theca Cell Culture Systems
- Oocyte In Vitro Maturation Assay

Implantation:

- Ishikawa cell test
- Human endometrial endothelial cells
- Human endometrial explants
- Placental perfusion
- Placental cells and cell lines

Prenatal development:

- Embryonal Carcinoma Cells
- Blastocyts and other Preimplantation Embryo Cultures
- Post implantation Whole Embryo Cultures (WEC)
- Embryonic Stem Cells (mouse and human)
- Transgenic Embryonic Stem Cells (including ReProGlo Assay)

Cross-cutting technologies:

- Sensor technologies
- Quantitative Structure-Activity Relationships (QSARs)
- Biotransformation
- Array technology
- Receptor interaction assays

From the outcome published in the international literature, the following method summaries are now or will be soon available in the DB-ALM database (February 2011):

- Computer Assisted Semen Analysis (CASA)
- SCSA and other in vitro Sperm DNA damage Assays
- Neutral Comet Assay in Sperm (ReProComet)

- Leydig Cell-enriched Cultures
- Sertoli Cell Enriched Cultures
- In Vitro Fertilisation Assay
- Follicle BioAssay (FBA)
- Granulosa and Theca Cell Culture Systems
- Oocyte In Vitro Maturation Assay
- Ishikawa cell test (including human endometrial endothelial cells and endometrial explants)
- Embryonal Carcinoma Cells
- Blastocyts and other Preimplantation Embryo Cultures
- Post implantation Whole Embryo Cultures (WEC)
- Embryonic Stem Cells (mouse and human)
- Transgenic Embryonic Stem Cells (including ReProGlo Assay)
- Placental perfusion
- Placental cells and cell lines (placenta-Cell Line function assay, trophoblast derived cell lines, pericytes, including placental primary cells)
- Quantitative Structure-Activity Relationships (QSARs)

The 14 most promising and successful tests, developed and/or optimised within the ReProTect project, where challenged as a test battery with 10 chemicals in the so-called “feasibility study”: The follicle Bioassay (FBA), the bovine *In Vitro* Maturation assay (IVM), the bovine *In Vitro* Fertilisation assay (IVF), the Mouse Embryonic Peri-Implantation Assay (MEPA), the Ishikawa cell test, the Whole Embryo Culture (WEC), the Embryonic Stem Cell Test (EST), the ReProGlo assay and several assays concerning endocrine disruption(receptor binding: ARBA, ERBA; luciferase activity tests: ER CALUX, AR CALUX, PALM, MELN) (Schenk *et al.*, 2010). Any of the 10 test chemicals was tested with these 14 assays, and the outcomes were compiled for the prediction of *in vivo* activity of the chemicals in the areas female fertility, male fertility and developmental toxicity. From there, three predictions were made for any of the 10 test chemicals: active or inactive in these three areas. 3 out of 30 predictions were partly correct, 4 out of 30 were incorrect and 23 predictions turned out to be correct when compared to*in vivo* results, which is rated as proof-of-principle (Schenk *et al.*, 2010). The authors expect a test battery approach like this to be very valuable in a tiered testing strategy for reproductive toxicity testing in future (Schenk *et al.*, 2010).

Status

The project was in progress from July 2004 and finished in December 2009.

Sponsors

European Commission

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Last update: February 2011