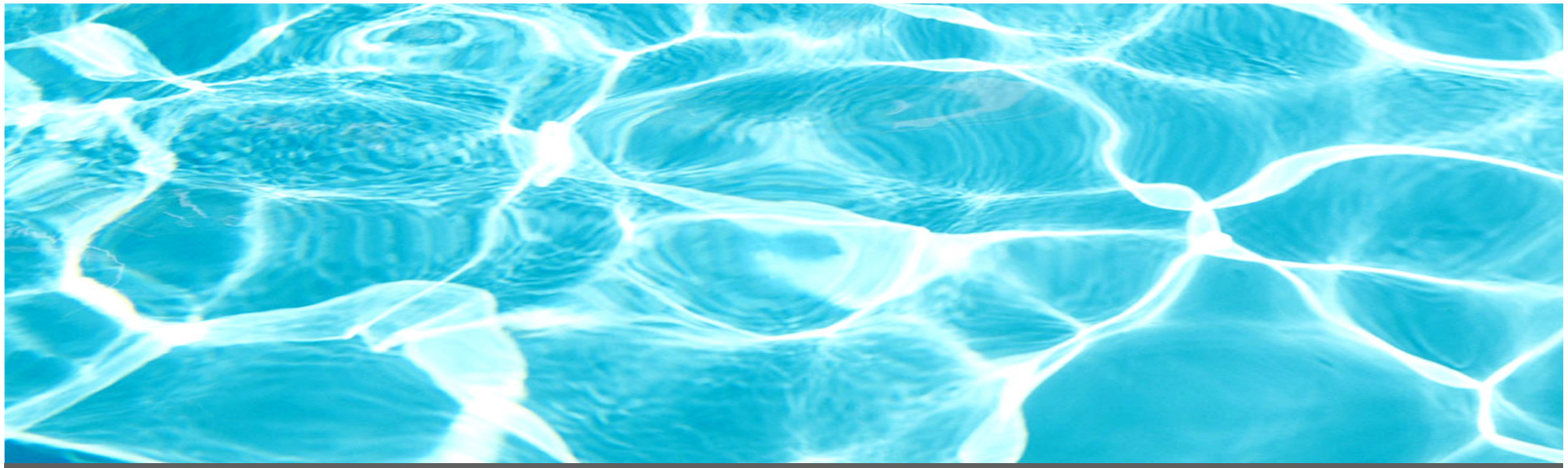


Alternative Methods for Safety Testing of Chemicals – Contributions from Industry

i-SUP, 25 April 2008

Marc Willuhn, *Cefic* *Research & Innovation*

cefic

10 YEARS LRI *The Long-range Research Initiative*

European Chemical Industry Council

Chemistry making a world of difference

- **Forum and voice of the chemical industry in Europe**
- **Brussels based office of 140 people working with over 4,000 people across the industry**

Cefic was founded in 1972

3R principles

- **Cefic is committed to the principles of**
  - **refinement**
  - **reduction**
  - **replacement**

  of animal testing

Long-range Research Initiative (LRI)

- **Mission**
  - Identify and fill gaps in our understanding of the hazards posed by chemicals
  - Improve methods available for assessing the associated risks
- **Strategic research**
  - Intelligent testing
  - Complex environments
  - Acceptance of technology
- **Value**
  - Network of experts
  - Engagement with scientific community
  - High-quality applied science

10 YEARS LRI The Long-range Research Initiative

www.cefic-lri.org

Long-range Research Initiative (LRI)

- **Research process:**
  - Regular publication of Request for Proposals (RfPs) – open to everyone
  - Requirement for researchers to publish findings in peer-reviewed journals
  - External Scientific Advisory Panel

- **Annual LRI Innovative Science Award for early-career scientists**
  - Since 2004
  - In cooperation with SETAC and EUROTOX
- **10th Annual LRI Workshop (Brussels, 20-21 Nov 08)**
  - Review of progress
  - Development of strategic research areas

10 YEARS LRI The Long-range Research Initiative

www.cefic-lri.org

Engagement

- **Cefic is founding member of EPAA (European Partnership for Alternative Approaches to Animal Testing)**
  - **Experts from member companies participating in Working Groups**
  - **Science and policy input**

- **EPAA Annual Conference**
  - 2008 Theme: research (Brussels, 4 Nov 2008)

Regulatory framework (REACH)

- **Goal: protection of human health and environment**
  - Duty of care
  - Legal requirements for safety testing

- **Promote development of alternative methods**
  - Currently few validated alternative methods available that suit regulatory needs and meet 3R criteria
  - Integrated testing strategies are needed

LRI projects

- **Refinement/reduction**
  - Testing strategies
  - Reprotoxicity studies
  - Databases
- **Replacement**
  - Computational tools
  - *In vitro* methods
  - Toxicogenomics

Testing strategies

- **Endocrine disruptors**
  - Validation of test methods (*e.g.* TG407, 21-day fish, Hershberger assay, non-castrated Hershberger assay, Uterotropic assay)
  - Development of Testing and Assessment Framework within OECD EDTA task force
  - Development of strategies for toxicology and ecotoxicology testing
- **Incorporation of categorisation and read-across**
- **Promotion of ITS**
  - European Technology Platform for Sustainable Chemistry (SusChem)
  - REACH Implementation Projects (RIPs)

OECD Conceptual Framework* for the Testing and Assessment of Endocrine Disrupting Chemicals

Industry-sponsored development and validation activities

Level 1

Sorting & prioritization based upon existing information

- physical & chemical properties, e.g., MW, reactivity, volatility, biodegradability,
- human & environmental exposure, e.g., production volume, release, use patterns
- hazard, e.g., available toxicological data

Level 2

***In vitro* assays** providing mechanistic data

- ER, AR, TR receptor binding affinity
- Transcriptional activation
- Aromatase and steroidogenesis *in vitro*
- Aryl hydrocarbon receptor recognition/binding
- QSARs
- High Through Put Prescreens
- Thyroid function
- Fish hepatocyte VTG assay
- Others (as appropriate)

Level 3

***In vivo* assays** providing data about single endocrine mechanisms and effects

- Uterotrophic assay (estrogenic related)
- Hershberger assay (androgenic related)
- Non -receptor mediated hormone function
- Others (e.g. thyroid)
- Fish screening assay

Level 4

***In vivo* assays** providing data about multiple endocrine mechanisms and effects

- enhanced OECD 407 (endpoints based on endocrine mechanisms)
- male and female pubertal assays
- adult intact male assay
- Fish sexual development assay
- Frog metamorphosis assay

Level 5

***In vivo* assays** providing data on effects from endocrine & other mechanisms

- 1-generation assay (TG415 enhanced)[1]
- 2-generation assay (TG416 enhanced)[1]
- reproductive screening test (TG421 enhanced)[1]
- combined 28 day/reproduction screening test (TG 422 enhanced)[1]

1 Potential enhancements will be considered by VMG mamm

- Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)

* Framework based on 6th meeting of OECD EDTA Task Force, see www.oecd.org

Reprotoxicity studies

- **Requirement under REACH for higher tonnages**
- **New design of mammalian reprotoxicity studies (1-generational vs 2-generational)**
  - Cooperation with EPAA, OECD and government bodies
  - Development of triggers/waivers by ECETOC Task Force
  - Co-sponsoring of expert workshop
  - Potentially saving 1200 animals per study
- **Research to develop genetically modified model organisms (signal transduction pathways) to increase predictivity**

Databases

- **BCF gold standard database**
  - Quality-controlled compilation of bioconcentration factors
  - Available under www.euras.be/bcf and ambit.acad.bg
- **RepDose**
  - Quality-controlled compilation of data from repeat-dose studies
  - Data-mining to study TTC concept

Computational tools

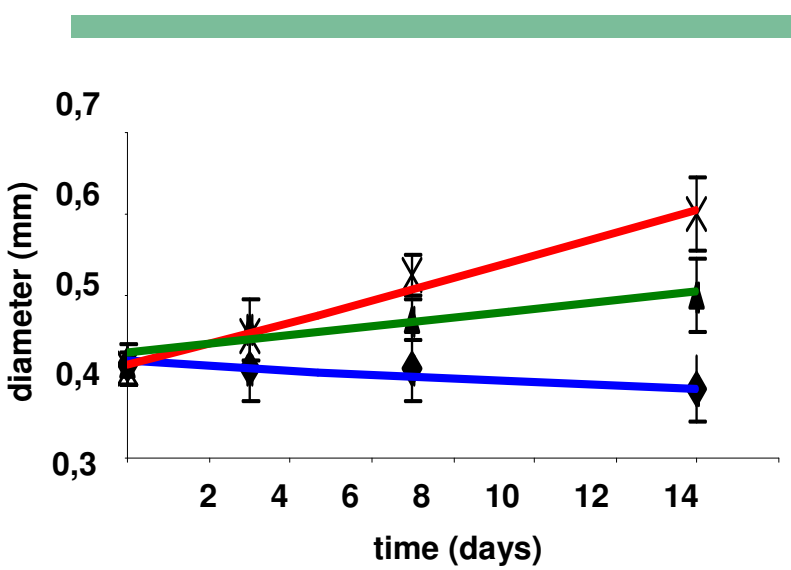
- **Categorisation and read-across can fill data gaps**
- **Development of tools**
  - OECD (Q)SAR Application Toolbox
  - AMBIT tool
  - PBPK database and model equation generator (HSL MEGen)
- **Acceptance of computational methods**
  - Funding of workshops (ICCA 2002, QSAR 2004)

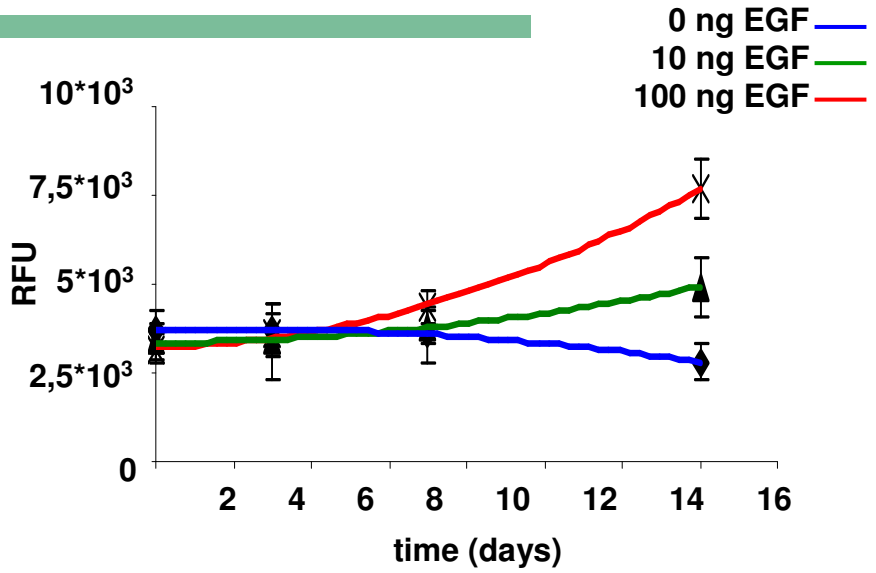
In vitro methods

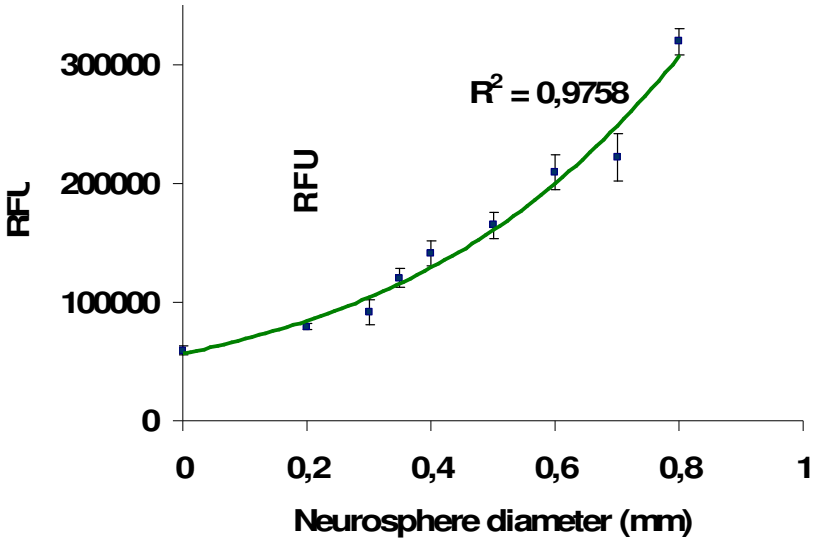
- **Development of *in vitro* methods**
  - Cell systems for DNT
    (LRI Innovative Science Award 2006)
  - Cell lines and fish embryos for acute and chronic toxicity

- **Co-sponsoring of workshops**
  - TestSmart DNT-II Symposium, 12-14 Nov 2008

Model: human neurospheres

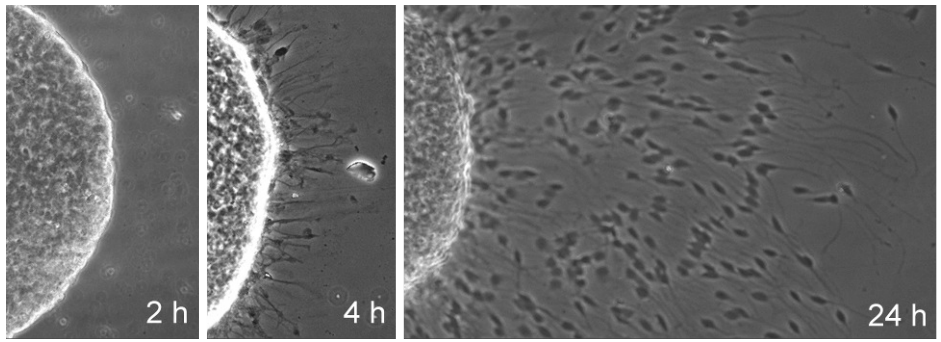


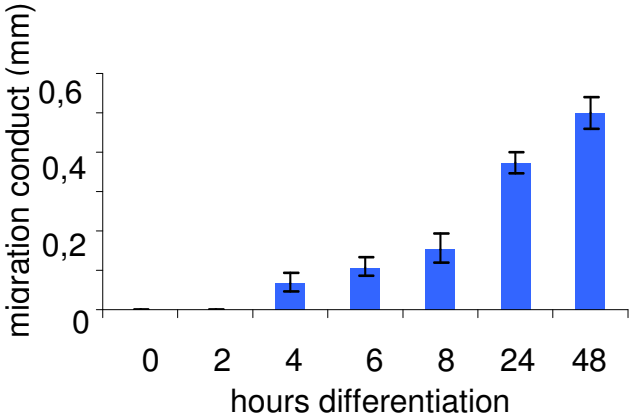




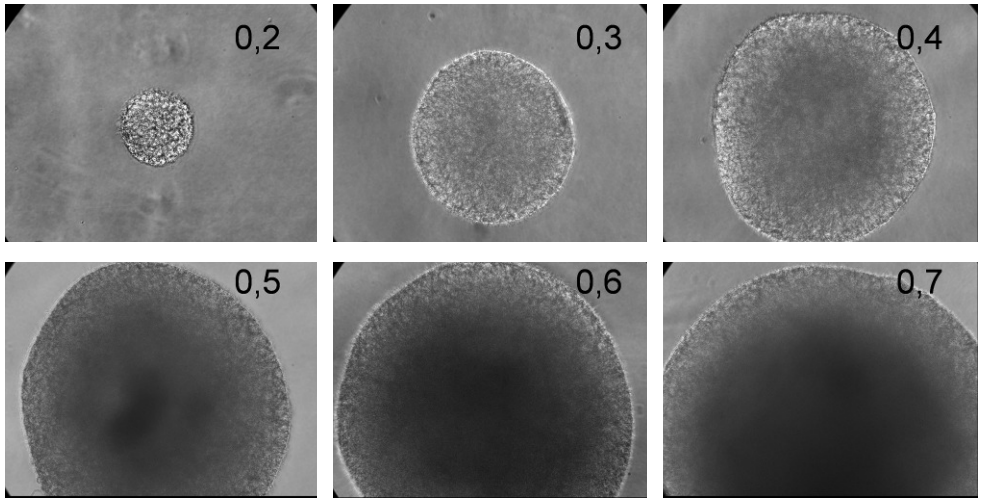
Moors *et al.*, *Environ. Health Perspect.*, in revision

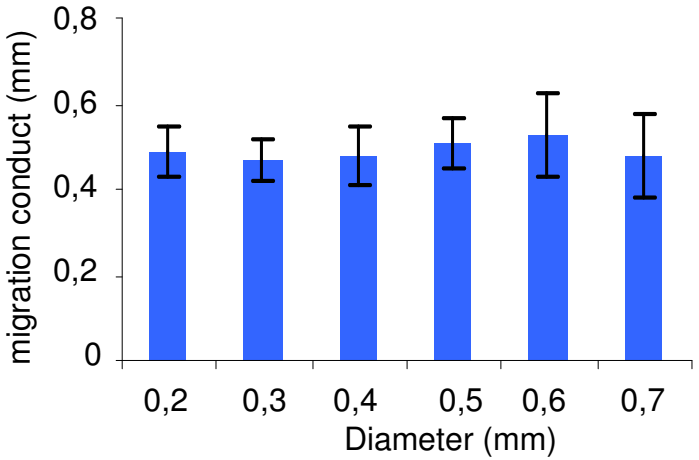
Human neurospheres – migration





Moors *et al.*, *TAAP* 2007





Moors *et al.*, *Environ. Health Perspect.,* in revision

Toxicogenomics

- **Use of new technologies in assessment of risk**
  - ICCA LRI Workshop (Amsterdam, 16-17 Jun 2008)
- **Research to improve understanding**
  - Background variation in gene expression
  - Identification of adverse effects
  - From data to information
  - Relevance for risk assessment and public health

Conclusions

- **Cefic committed to 3Rs concept**
- **Progress with 3Rs, focus on 1R alone can cause delays**
  - For complex endpoints, replacement is a long-term goal
  - In the short term, refinement and reduction will have a higher impact on animal numbers
- **Cefic engaged in practical work through various research projects**
- **Successful development and acceptance of alternative methods requires**
  - Early involvement of regulators and relevance for regulatory questions
  - Taking into account relevant legislation
  - Integration into an overall testing strategy

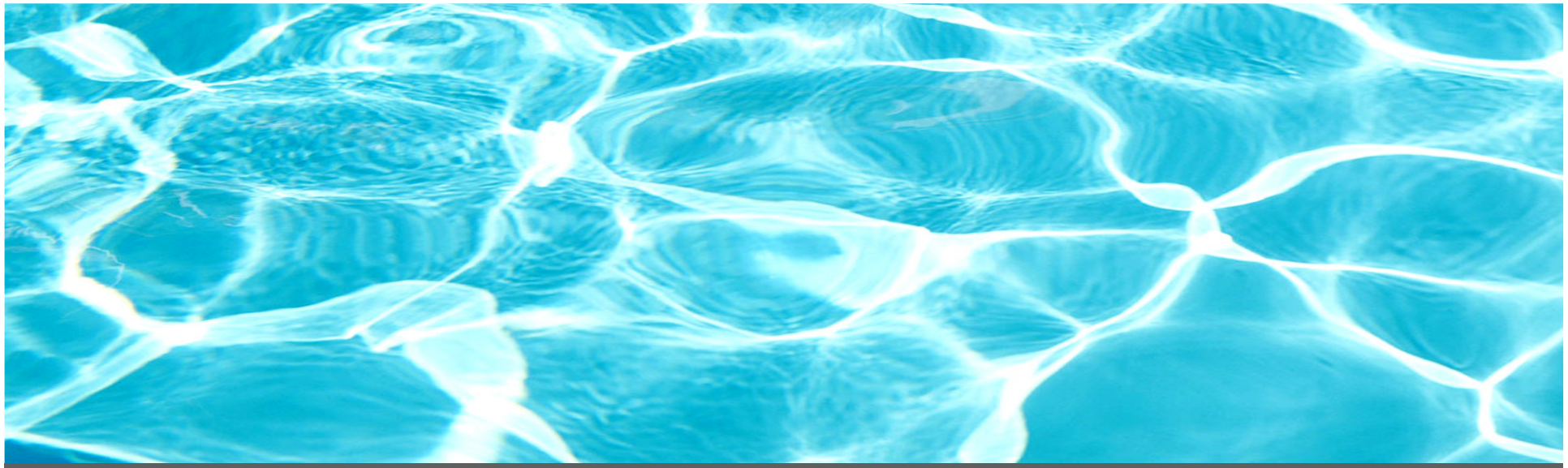
- **Trade-off between animal welfare and safety?**

3R principles

Refinement

Reduction

Replacement



Alternative Methods for Safety Testing of Chemicals – Activities of Industry

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LRI
The Long-range Research Initiative

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LRI projects

- **Selection of LRI projects relevant to animal alternatives**
- **More projects in other areas**
  - DNA adducts and chemical carcinogenesis
  - Immunotoxicity and allergies
  - Male reproductive health
  - Exposure models
  - Environmental databases and modelling
  - Persistency and bioaccumulation

LRI completed projects

- ***Gene expression profiling*** **(CC2)**
  Harry van Steeg, Leiden University **$648,000**
- ***PBPK MeGEN*** **(B3.7)**
  George Loizou, HSL **€302,000**
- ***Identification of SAR Alerts for Substances with Low NOELs – RepDose database*** **(B1.1)**
  Inge Mangelsdorf, Fraunhofer **$212,000**
- ***Review of the Predictive Power of One-generational versus Multi-generational Studies to Detect Endocrine Disruptive Activity of Chemicals*** **(EMSG41)**
  Paul Harrison, Cranfield University **€58,000**

LRI ongoing projects

- ***Model organisms with genetically sensitized signal transduction pathways as predictors for mammalian developmental toxicity*** **(AIMT1)**
  George Daston, P&G **€200,400**
- ***Development of a reference database for bioconcentration factors*** **(ECO7)**
  Bram Versonnen, EURAS **€48,500**
- ***Development of a strategy to predict acute fish lethality using fish cell lines and fish embryos*** **(ECO8)**
  Kristin Schirmer, UFZ Leipzig **€400,000**
- ***QSAR Software – OECD Toolbox***
  Bob Diederich, OECD **€150,000**
- ***QSAR Software – AMBIT*** **(EEM9)**
  Joanna Jaworska, P&G
  Nina Jeliazkova, IdeaConsult **€150,000**
- ***Finalisation and extension of RepDose database*** **(B1.1.3, B1.7)**
  Inge Mangelsdorf, Fraunhofer **€164,500**

LRI new projects

- ***Use of REPDOSE for evaluation/refinement of the TTC-concept*** **(B1.8)**
  Inge Mangelsdorf, Fraunhofer €55,000
- ***Initial explorations on alternatives for the fish chronic toxicity test using fish cell lines, embryos*** **and "omics" (ECO8.2)**
  Kristin Schirmer, UFZ Leipzig €60,000
- ***Male Reproductive Health and Endocrine Toxicity: Application of Toxicogenomic Technologies to Develop a Mechanistic-Based Risk Assessment*** **(EMSG46)**
  ca. €1,300,000
- ***Support of a tiered approach to evaluate endocrine effects in aquatic organisms*** **(EMSG47)**
  ca. €450,000