Meta Analysis of a Battery Test of Reproductive Toxicity Assays

The RePro Tect Experience

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2004-2009

(www.reprotect.eu)

Development of a novel approach in hazard and risk assessment of reproductive toxicity by a combination and application of in vitro, tissue and sensor technologies

- **Integrated project funded through the EU FP6 program**
- **Total budget amounts: 13.2 mEUR**

LSHB-CT-2004-503257

Structure of the ReProTect Project

Open Tox, Munich, August 9-12, 2011

Bayer HealthCare

EggCentris
Center of Excellence for Reproductive Function Testing

Istituto Sperimentale Italiano "Lazzaro Spallanzani"

IT University of Copenhagen

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Imperial College

33 partners

from Academia, Industry, SMEs and Governmental Institutes

BDS
BioDetection Systems

Vrije Universiteit Brussel

ProteoSys
Innovation in Functional Proteomics

UPPSALA UNIVERSITET

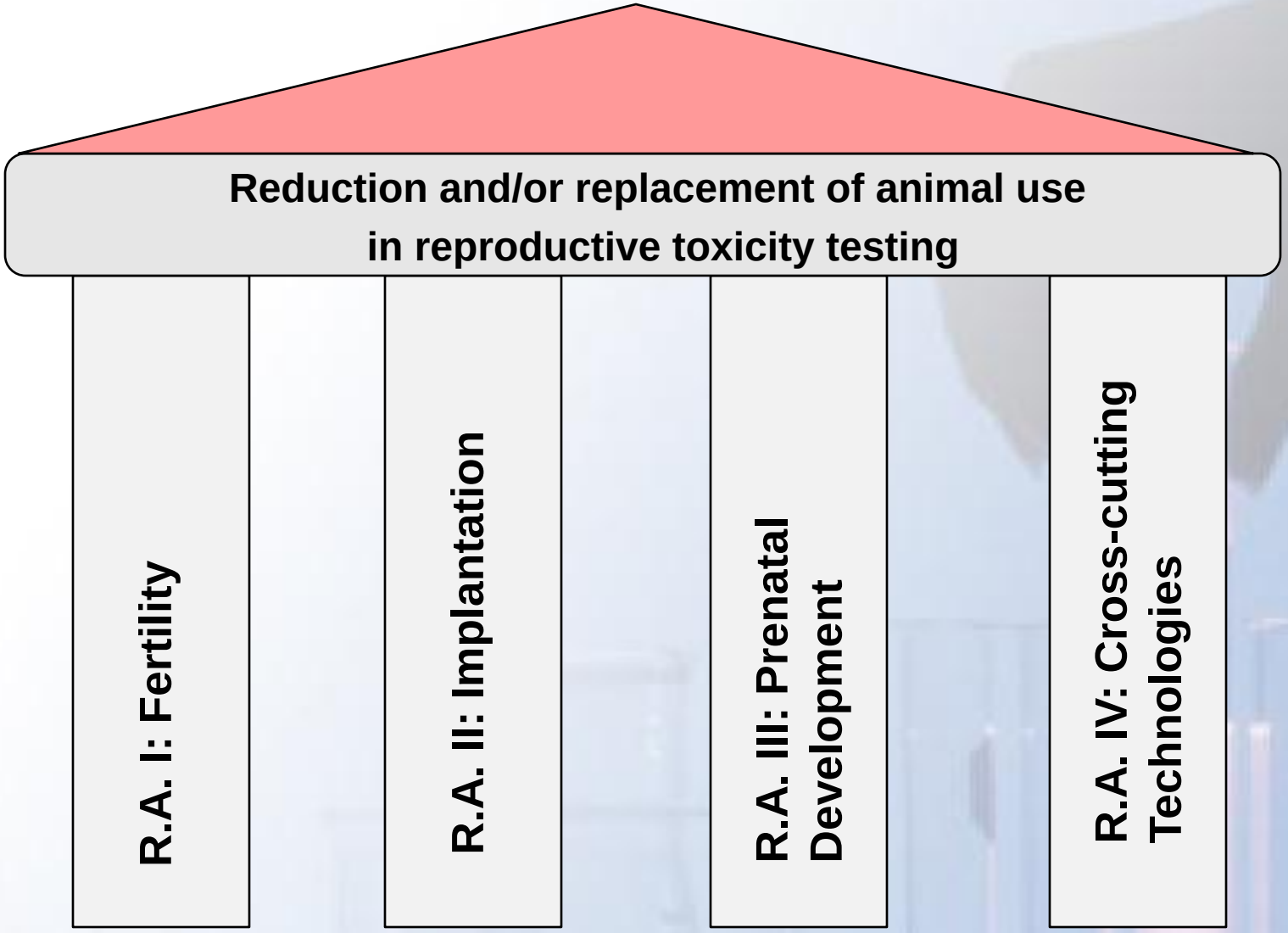
vito
vision on technology

Universität Konstanz

Johnson & Johnson
PHARMACEUTICAL RESEARCH & DEVELOPMENT

Structure of the ReProTect Project

Open Tox, Munich, August 9-12, 2011



Coordination:
M. Schwarz, Tübingen

Project management (Financial):
S. Stoppel, Tübingen

(Scientific)
S. Bremer, ECVAM, Ispra
C. Pellizzer, ECVAM, Ispra

Advisory Board (chair):
Bernward Garthoff

Research Area leaders:
I.: G. Lazzari, Cremona
II: L. Dencker, Uppsala
III: H. Spielmann, Berlin
IV: A. Mantovani, Rome

Specific Endpoints in the Reproductive Cycle

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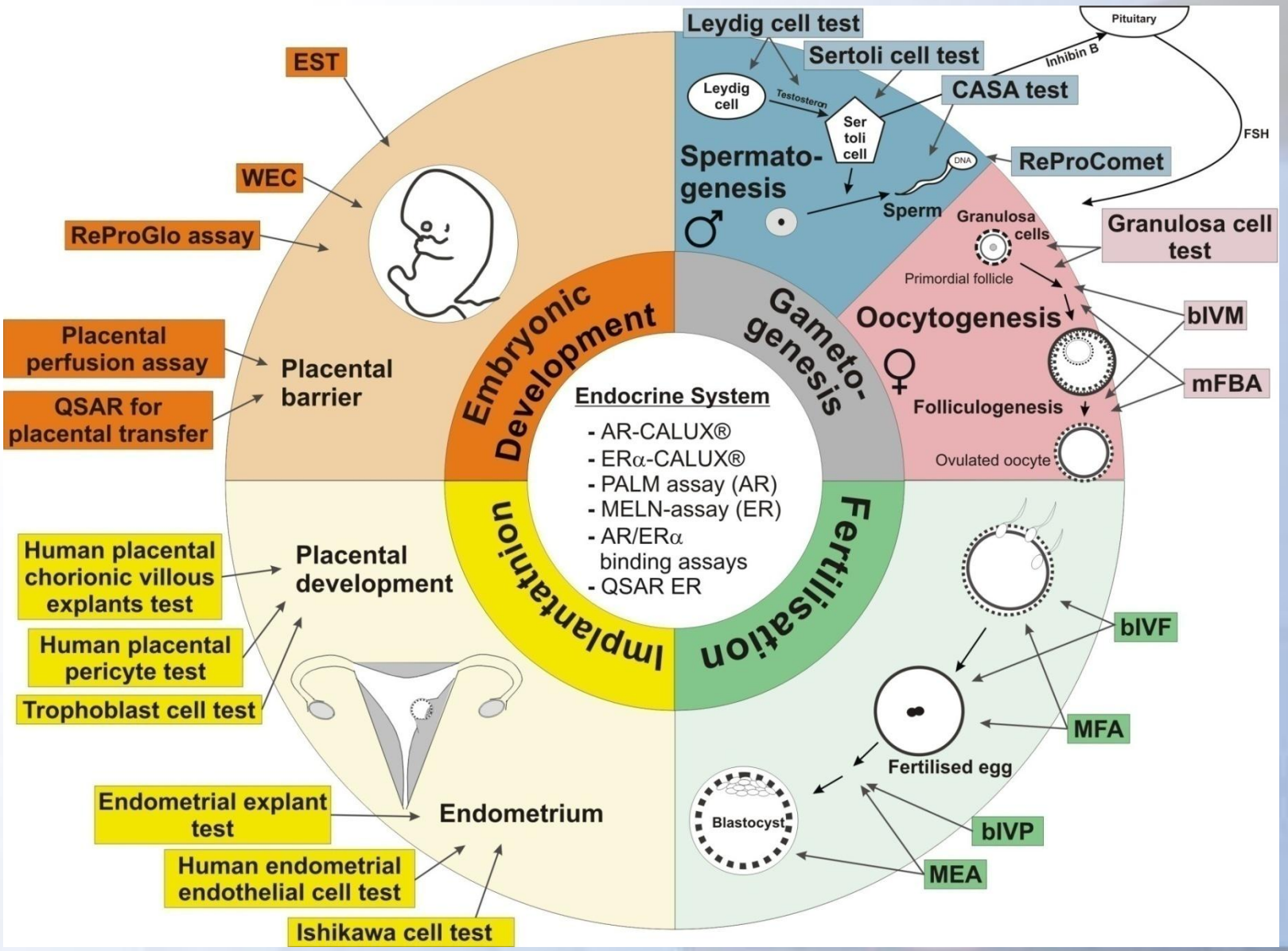


The ReProTect database

Open Tox, Munich, August 9-12, 2011

Server: localhost ▸ Datenbank: ReProTect

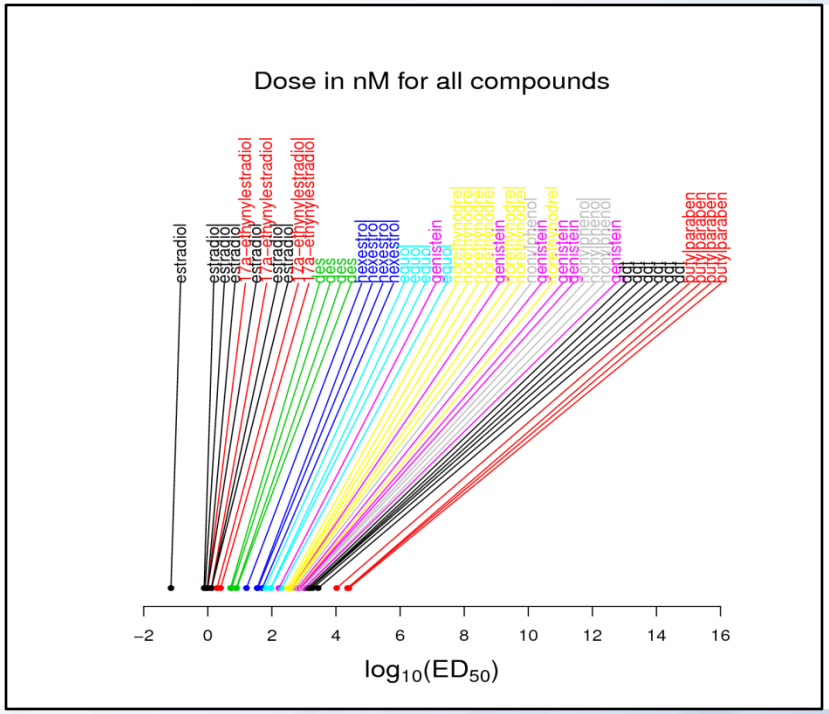
Struktur | SQL | Suche | Abfrageeditor | Exportieren | Importieren | Operationen | Rechte | Löschen

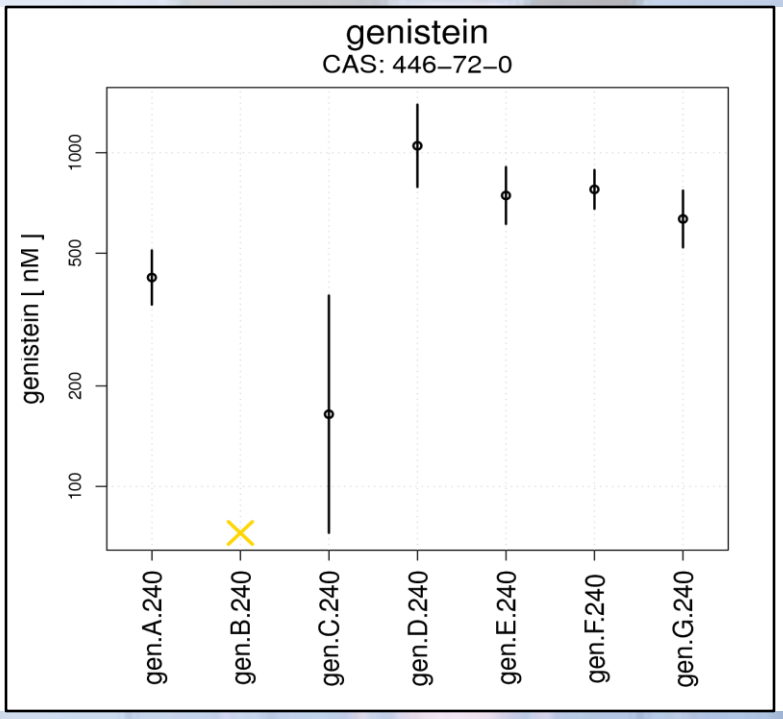
	Tabelle	Aktion	Einträge	Typ	Kollation	Größe	Überhang
☐	Assay		~28	InnoDB	utf8_general_ci	48,0 KiB	-
☐	CompoundHarm		~128	InnoDB	utf8_general_ci	64,0 KiB	-
☐	CompoundInd		~155	InnoDB	utf8_general_ci	32,0 KiB	-
☐	DoseUnit		~6	InnoDB	utf8_general_ci	32,0 KiB	-
☐	ED50		~890	InnoDB	utf8_general_ci	96,0 KiB	-
☐	Endpoint		~25	InnoDB	utf8_general_ci	32,0 KiB	-
☐	Lab		~15	InnoDB	utf8_general_ci	16,0 KiB	-
☐	Run		~1,522	InnoDB	utf8_general_ci	336,0 KiB	-
☐	Target		~7	InnoDB	utf8_general_ci	16,0 KiB	-
☐	Treatment		~338	InnoDB	utf8_general_ci	96,0 KiB	-
☐	WarningsHarm		~8	InnoDB	utf8_general_ci	32,0 KiB	-
☐	WarningsInd		~632	InnoDB	utf8_general_ci	96,0 KiB	-
	12 Tabellen	Gesamt	~3,754	MyISAM	latin1_swedish_ci	896,0 KiB	0 Bytes

Alle auswählen / Auswahl entfernen markierte:

GlobalID	Assay	Target	Endpoint	Lab	Compound	CAS	Run	ED50
670		6	Standard		genistein	446-72-0	gen.A.240	422.6
672		6	Standard		genistein	446-72-0	gen.C.240	164.5
673		6	Standard		genistein	446-72-0	gen.D.240	1049.0
674		6	Standard		genistein	446-72-0	gen.E.240	744.5
675		6	Standard		genistein	446-72-0	gen.F.240	776.4
676		6	Standard		genistein	446-72-0	gen.G.240	633.1

Lower	Upper	DoseUnit	MW	CompoundHarm	CompoundRef	RefED50	RefDoseUnit
350.2	509.9	nM	270.2369	genistein	genistein	4.226e-07	M
72.5	373.4	nM	270.2369	genistein	genistein	1.645e-07	M
788.8	1394.0	nM	270.2369	genistein	genistein	1.049e-06	M
611.3	906.6	nM	270.2369	genistein	genistein	7.445e-07	M
678.6	888.4	nM	270.2369	genistein	genistein	7.764e-07	M
520.1	770.7	nM	270.2369	genistein	genistein	6.331e-07	M





~150 compounds

Tests for Detection of (anti-)Endocrine Activity

Open Tox, Munich, August 9-12, 2011

Bart van der Burg — BDS BioDetection Systems

Hilda Witters — vito vision on technology

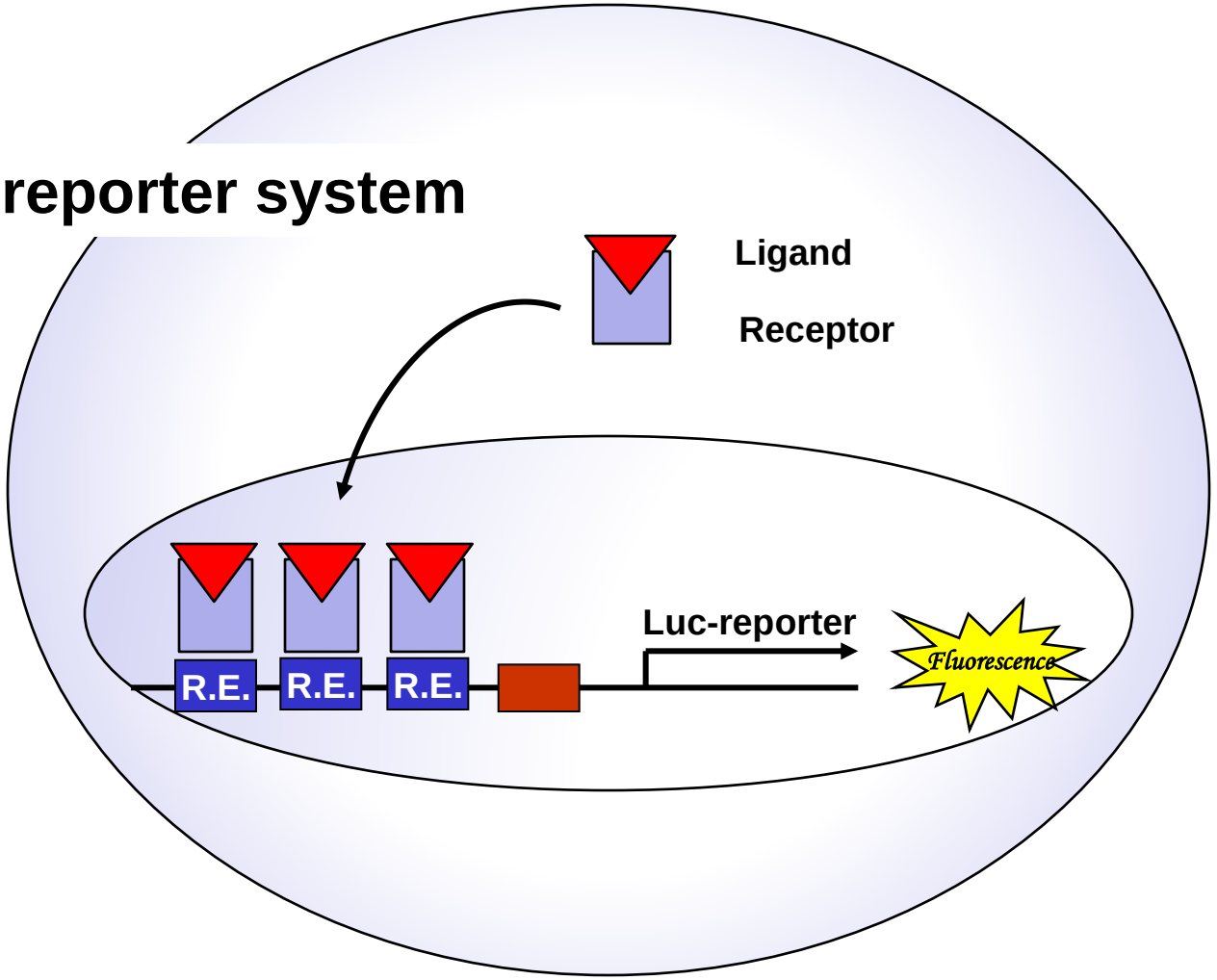
Alexius Freyberger — Bayer HealthCare, Bayer Schering Pharma

Ovanes Mekenyan

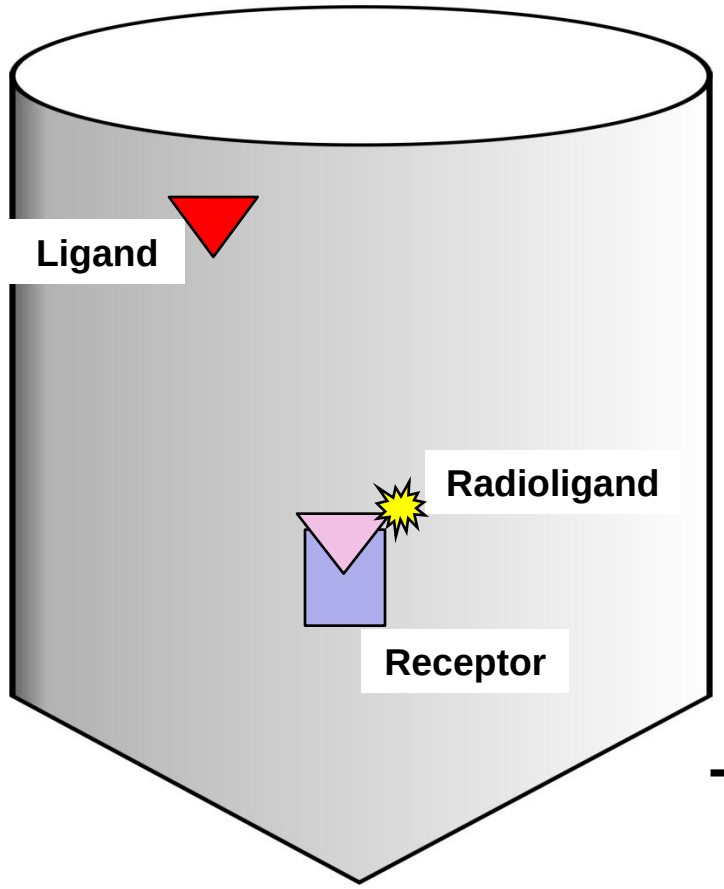
Endocrine System

- AR-CALUX®
- ERα-CALUX®
- PALM assay (AR)
- MELN-assay (ER)
- AR/ERα binding assays
- QSAR ER

Cell-based reporter system



Receptor Binding Assay

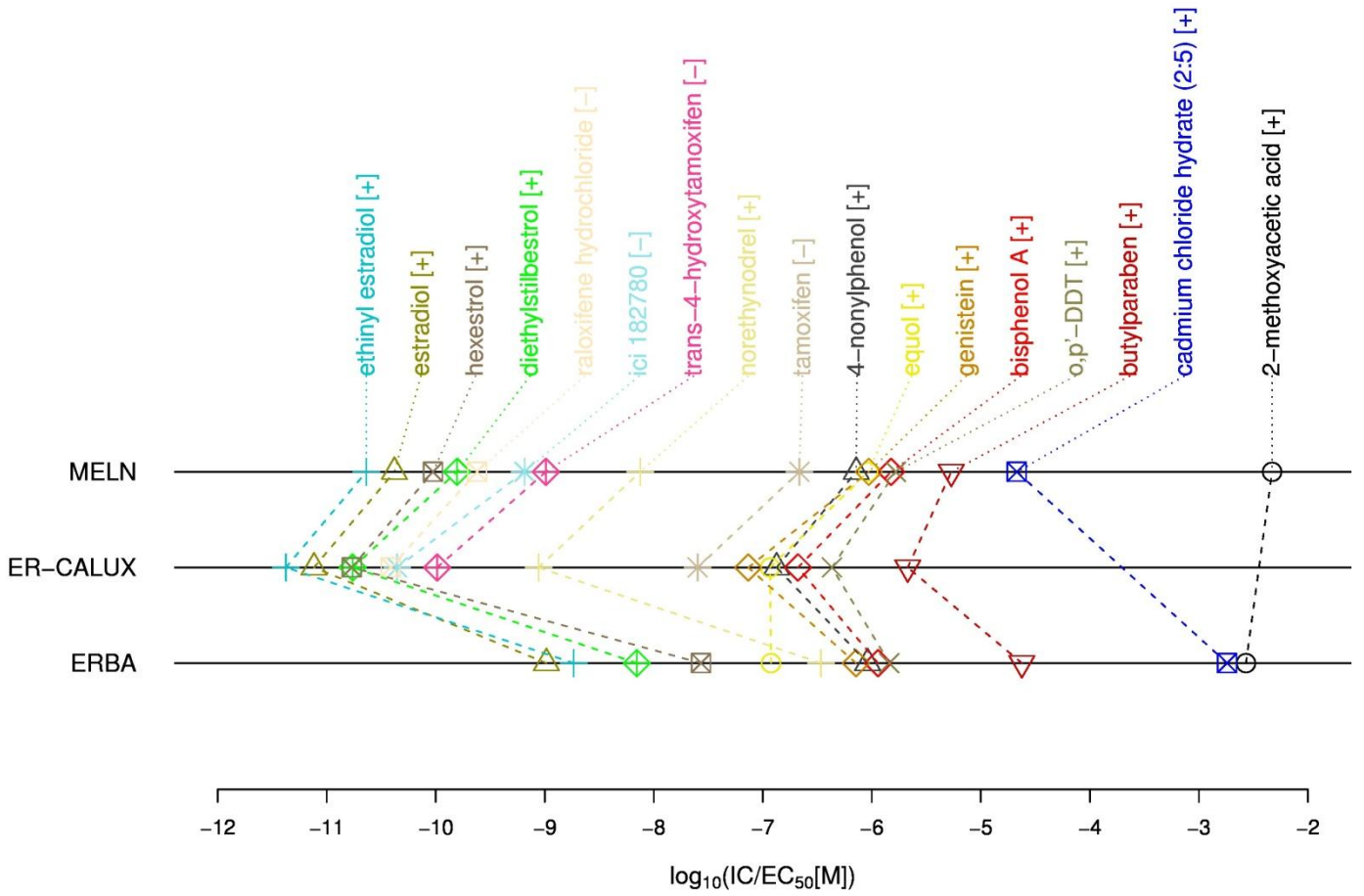


Test tube

(Anti-)Estrogenic Effects: Comparison of *in vitro* assays

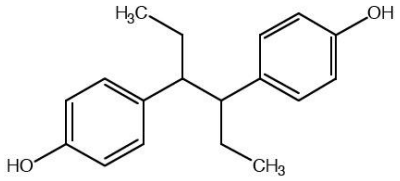
Cell-based reporter assays

Receptor binding assay

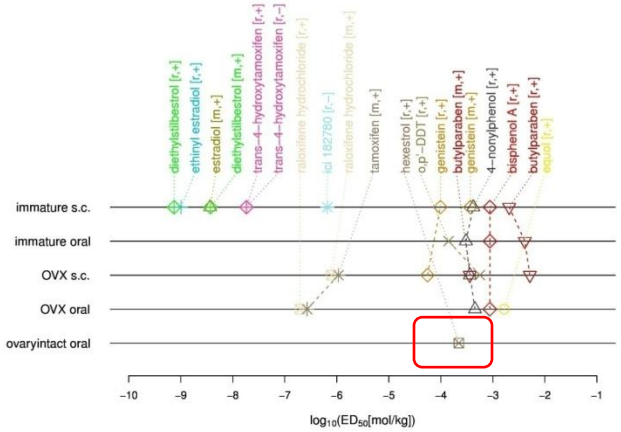


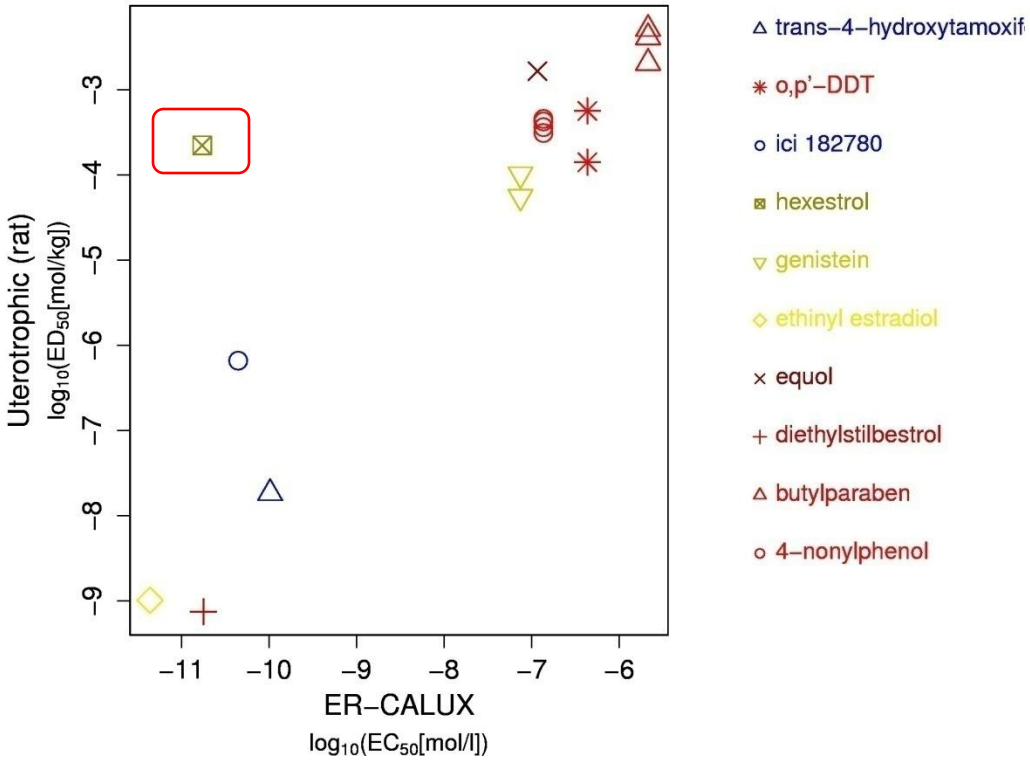
(Anti-E)estrogenic Effects: Comparison *in vitro* / *in vivo*

Hexestrol



Oral application



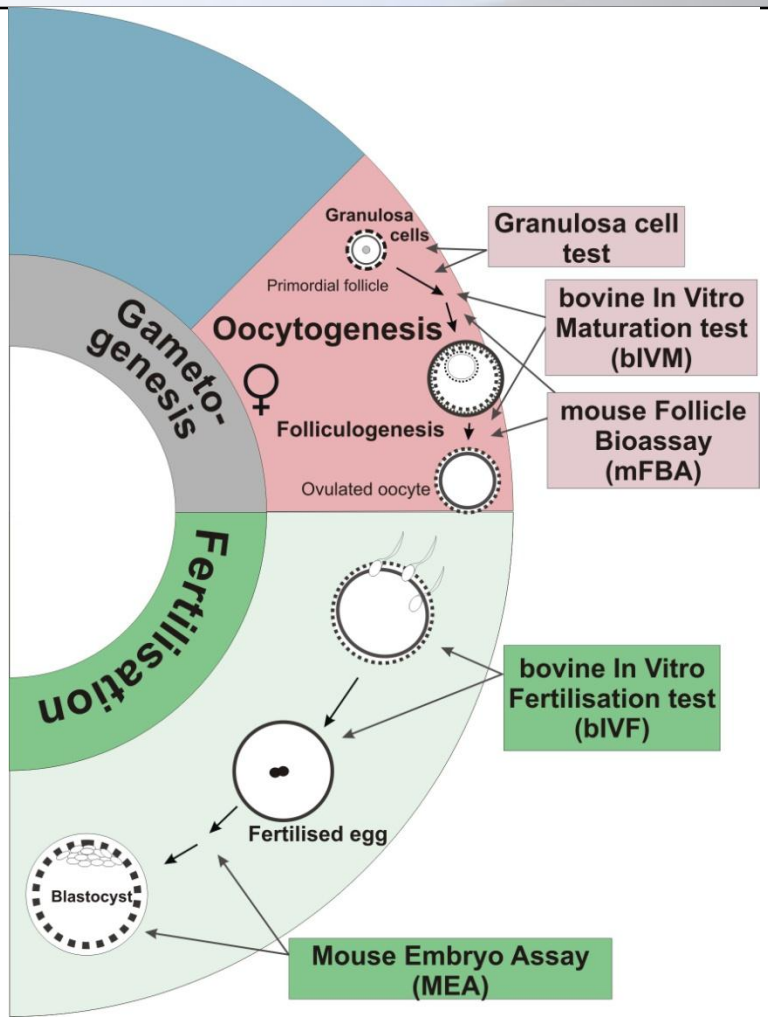


Female Fertility

Open Tox, Munich, August 9-12, 2011

Giovanna Lazzari

Rita Cortvrindt



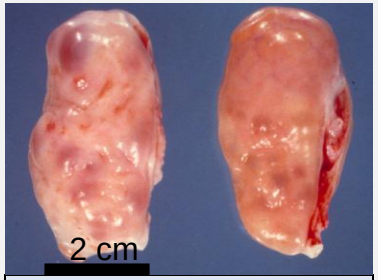
Use of Bovine Gametes for Reproductive Toxicity Testing

Open Tox, Munich, August 9-12, 2011

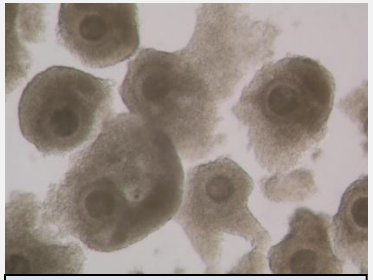
Giovanna Lazzari

AVANTEA
LABORATORIO DI TECNOLOGIE DELLA RIPRODUZIONE

In vitro **maturation** (IVM) of bovine oocytes

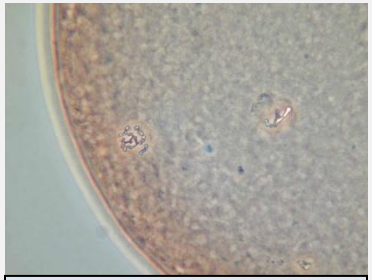


bovine ovaries



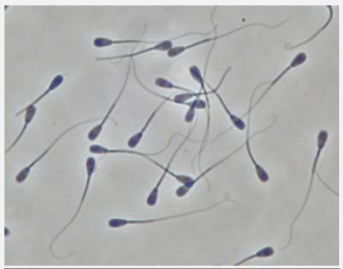
bovine oocytes

Chemical exposure (24h) during IVM

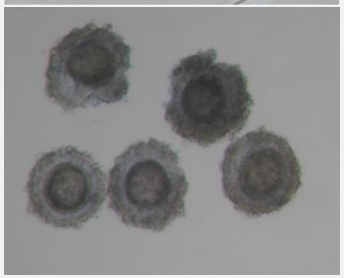


Endpoint:
% Metaphase II

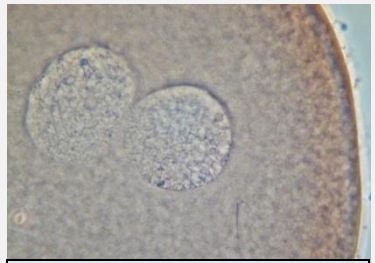
In vitro **fertilisation** (IVF) of bovine oocytes



IVM, 24 h



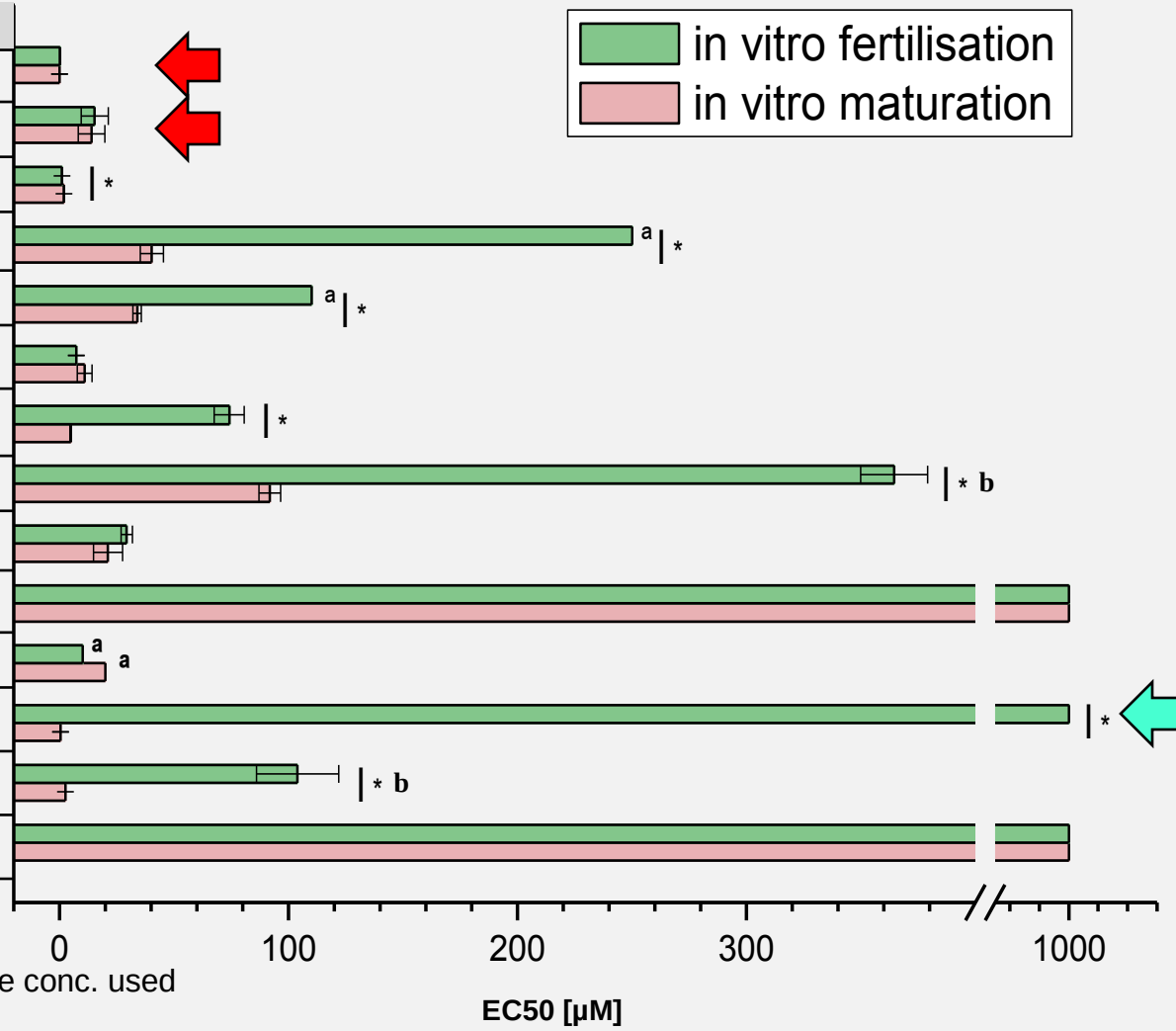
Chemical exposure (20h) during IVF



Endpoint:
% 2Pronuclei

Results obtained with the bIVM and bIVF Bioassays

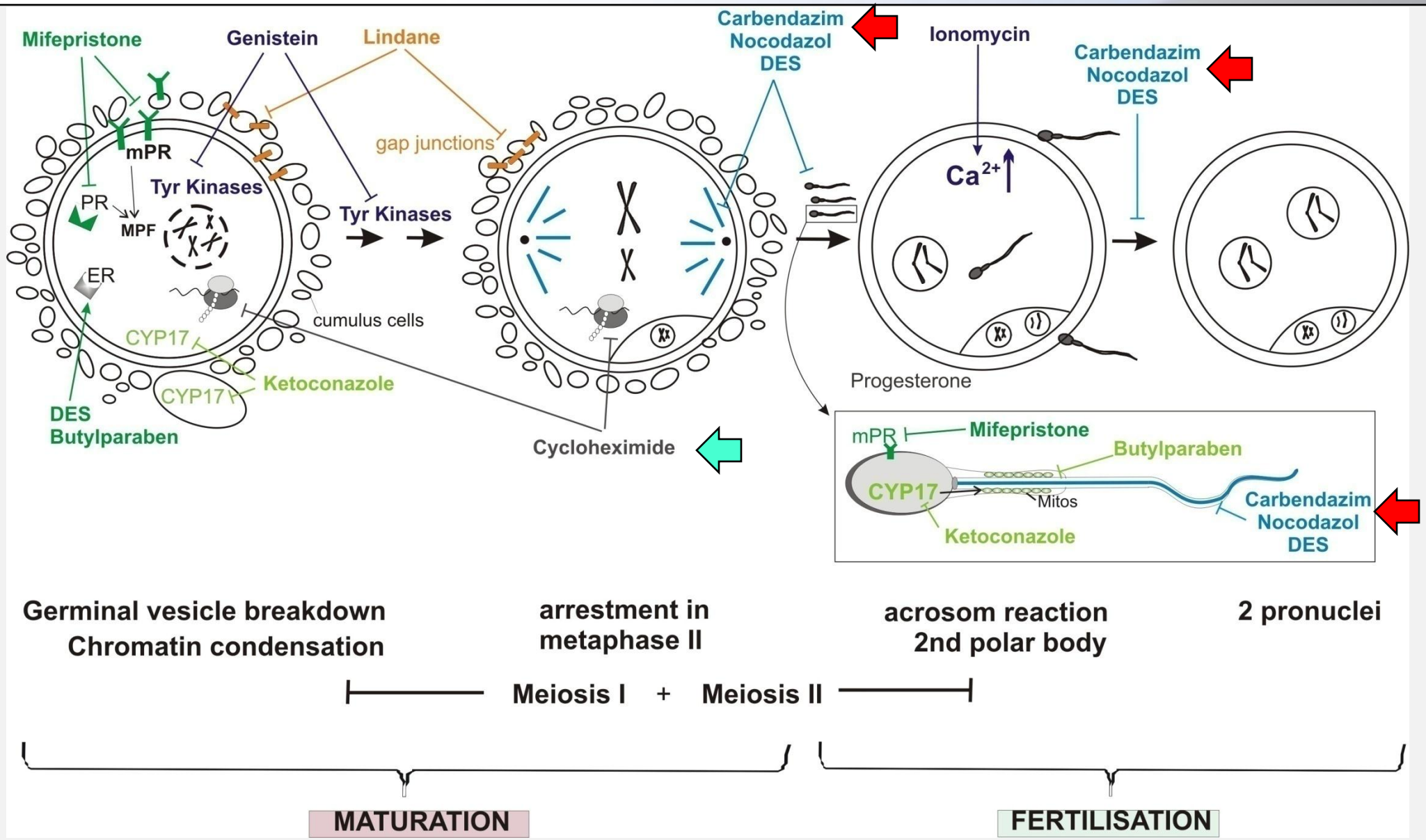
Molecular Target	Effect	Chemical
Mikrotubuli	Aneuploidy-inducing	Nocodazol
	Aneuploidy-inducing	Carbendazim
Signal-transduction	Calcium ionophor	Ionomycin
	Tyr-Kinase inhibitor	Genistein
Gap junctions	Weakly estrogenic, GJC inhibitor	Lindane
Endocrine System	Anti-progestogen	Mifepristone
	Estrogenic	Diethylstilbestrol
	Weakly estrogenic	Butylparaben
	Steroidogenesis inhibitor	Ketoconazol
DNA	Clastogen	Busulfan
	Mutagen	Benzo[a]pyren
Ribosom	Protein synthesis inhibitor	Cycloheximide
	General toxicant	$CdCl_2$
	Unknown	Methyl acetoacetate



[a] Solubility limit reached
[b] Cytotoxic to gametes at the conc. used

Modified from: G. Lazzari et al., TAP 233, 360 (2008)

Suggested Mechanisms of Compounds



Embryotoxicity Tests

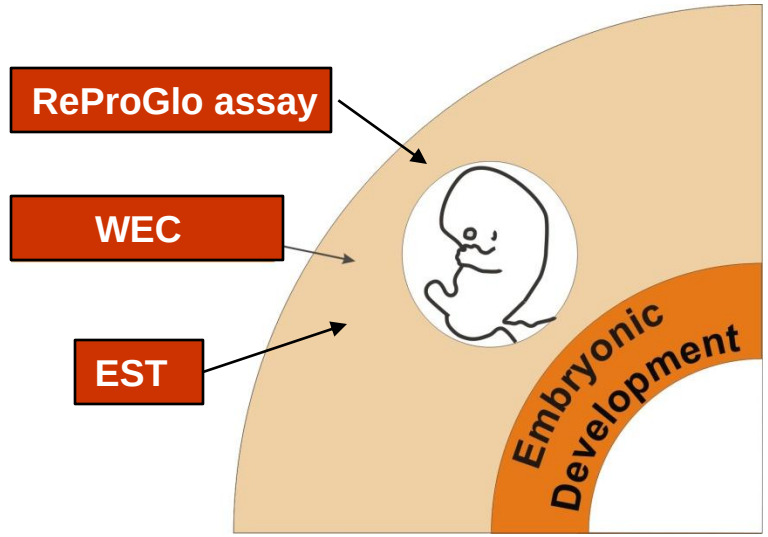
Open Tox, Munich, August 9-12, 2011

Horst Spielmann,
Andrea Seiler

Aldert Piersma

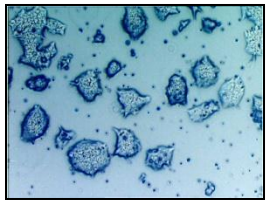
Michael Schwarz

Heinz Nau

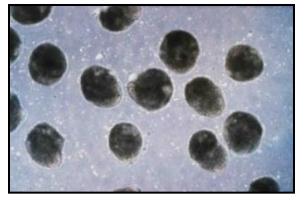


Embryotoxicity Tests

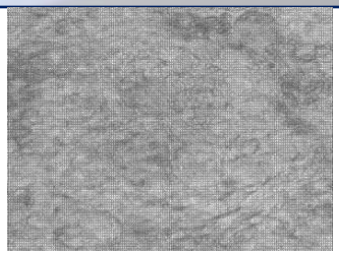
EST



day 0: undifferentiated mouse-ES-cells



day 3-5: *in vitro* differentiation to „embryonic bodies “



day 5-10: *in vitro* differentiation to contracting heart muscle cells

Incubation with test chemical

ReProGlo

Wnt/TCF-reporter-transfected mES cells

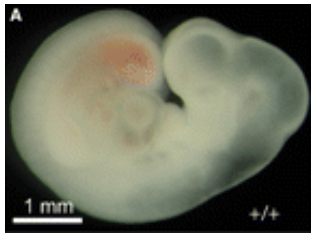
Alamar blue (cytotoxicity)

Fluorescence

Test chemical

0 24 46 48 (h)

Whole Embryo Culture (WEC)

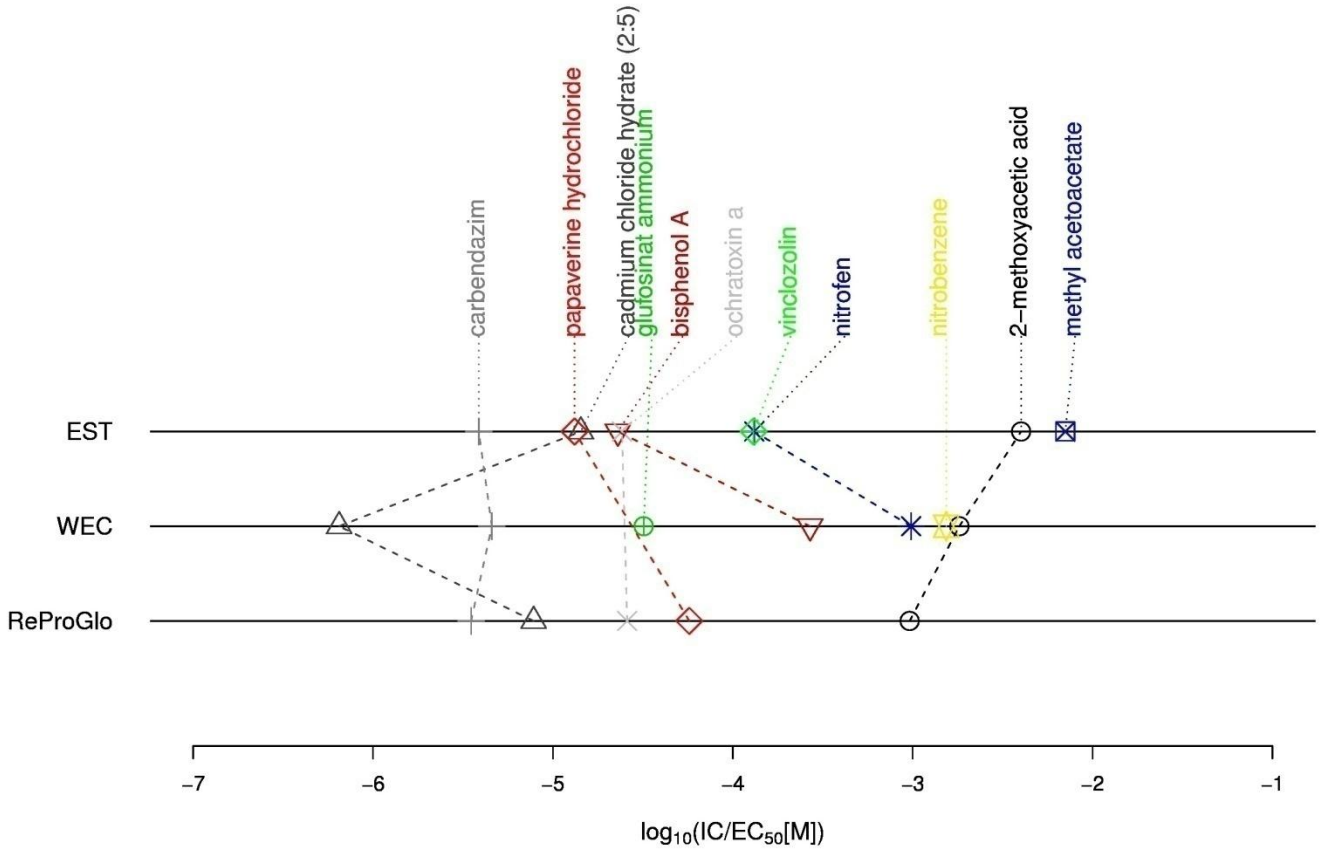


Embryo, day 9,5-11,5

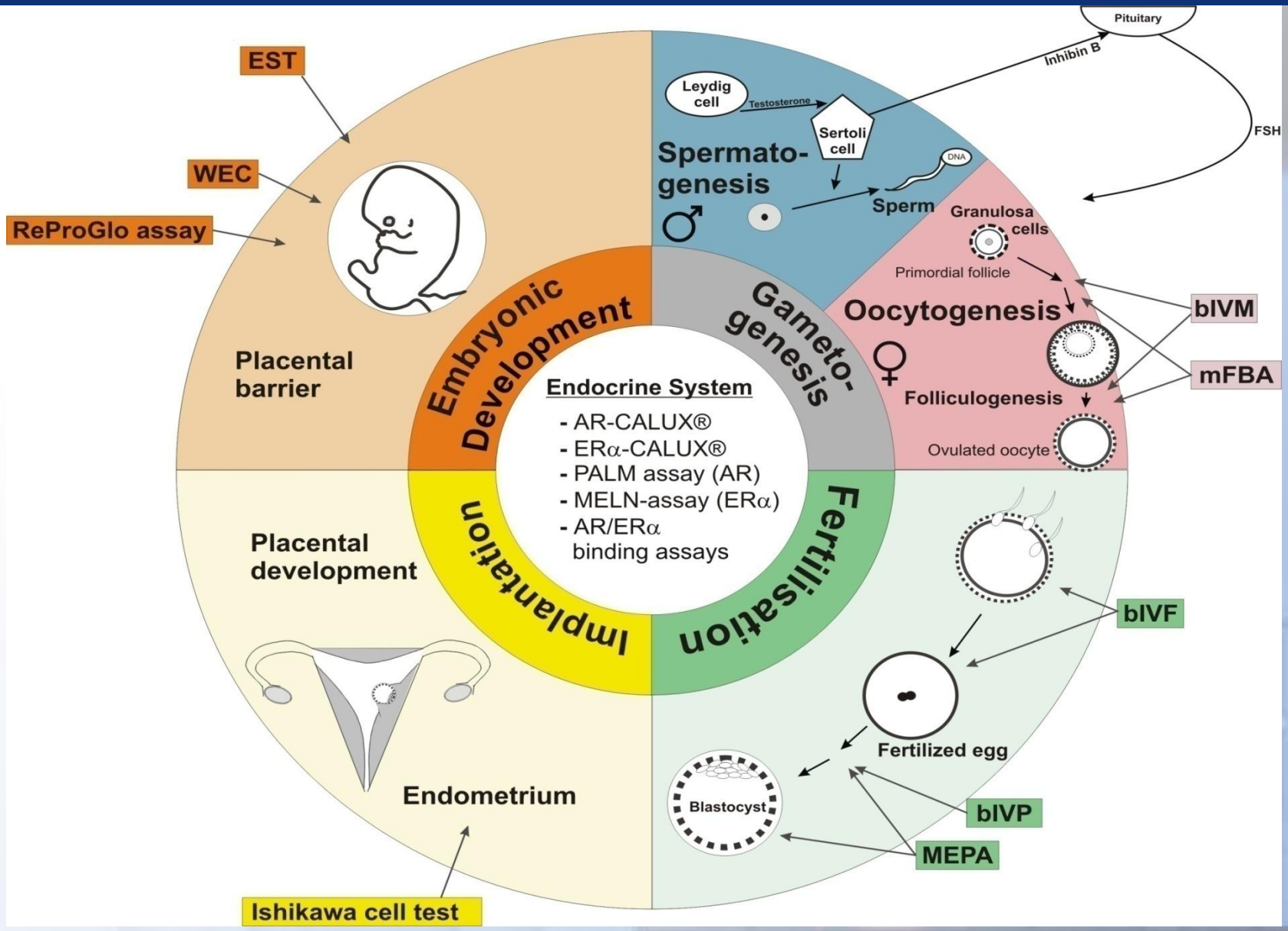
Incubation with test chemical

48h - max. 96h

Embryotoxicity Tests



The ReProTect Feasibility Study



The ReProTect Feasibility Study

~150 ReProTect chemicals

Selection of 10 chemicals by independent experts

Selection criteria:

- *In vivo* effects well characterized
- No metabolic activation (CYP450-mediated) required

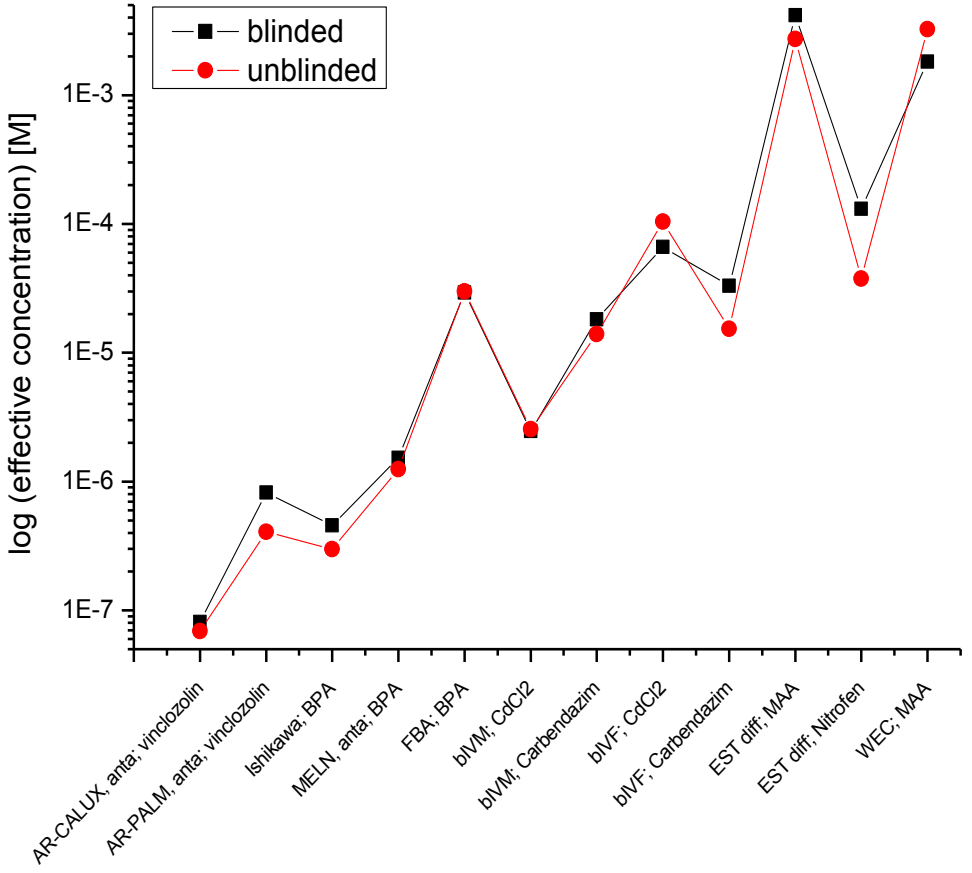
Blinding of chemicals (chemical 1,2,3,...10) and distribution to the experimental groups

Determination of EC_{50}-(or equivalent) values for all 10 chemicals in each of the assays

The ReProTect Feasibility Study

Open Tox, Munich, August 9-12, 2011

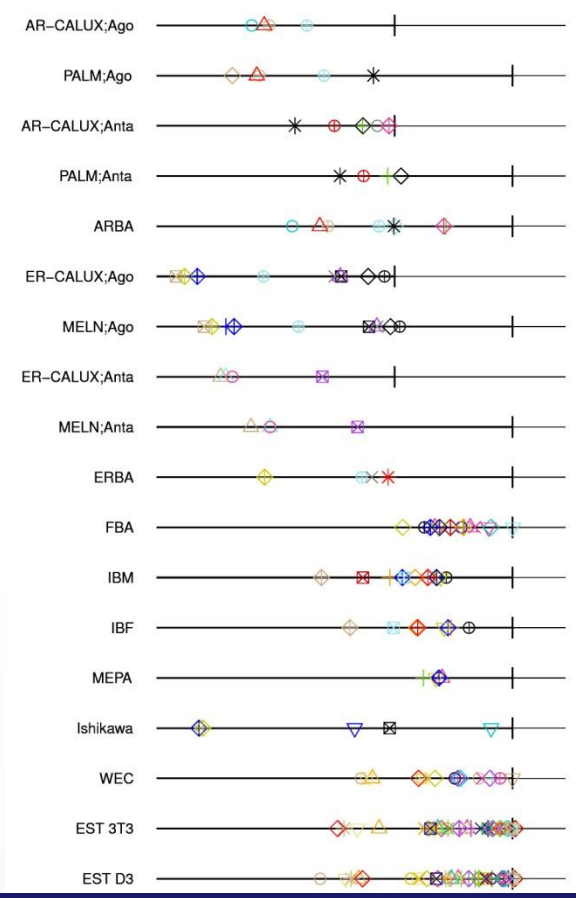
TEST	Known reference compounds out of the 10 test compounds
ARBA	1 / 10
AR -CALUX	1 / 10
AR-PALM	1 / 10
ERBA	- / 10
ER -CALUX	- / 10
MELN	- / 10
FBA	- / 10
bIVM	3 / 10
bIVF	3 / 10
MEPA	- / 10
Ishikawa cell test	1 / 10
WEC	1 / 10
EST	2 / 10
ReProGlo	- / 10



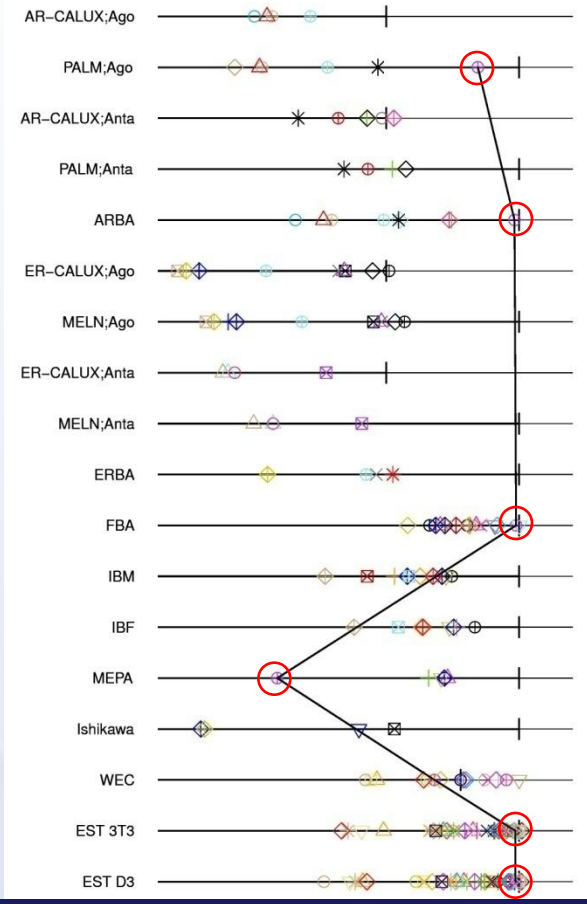
The RePro Tect Feasibility Study

Open Tox, Munich, August 9-12, 2011

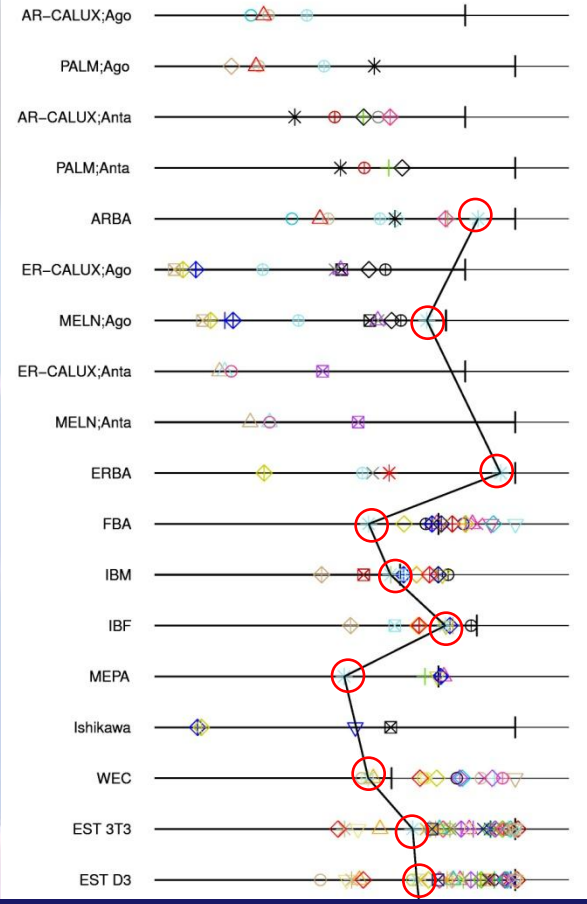
Sodium chloride



Glufosinate Ammonium



Cadmium chloride



- **Nearest neighbor analysis** (Prediction models, if available, were NOT used)
- **Weight of evidence approach** (Expert knowledge)

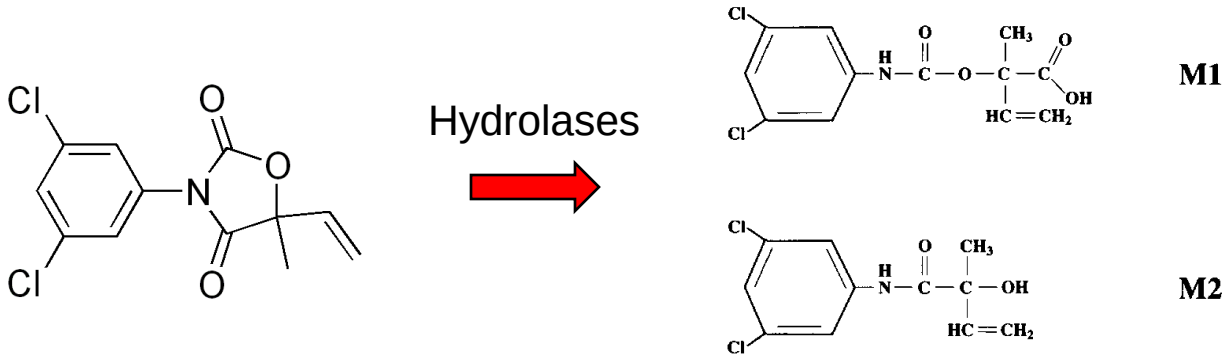
Predictions from the Feasibility Study

1	This water-soluble chemical is negative in all assays except in the FBA where it is positive at very high concentrations.	We regard chemical 1 as having a low potency. We do not expect that this compound would affect female fertility or -if at all- at high dose levels only. We do not expect the compound to cause embryotoxicity *in vivo*.
2	This water-soluble chemical is negative in the receptor binding/activation assays (effects at very high concentrations are regarded as irrelevant). The compound is active at a very low concentration (~$5x10^{-7}$ mg/ml) in the MEPA assay. We therefore predict that the compound negatively affects morulation or blastocyst formation. The compound is unsuspicious in all other assays that predict effects on female fertility. Compound 2 had no effect on D3 differentiation and is only toxic for 3T3 and D3 cells at a very high concentration. It is also negative in the ReProGlo assay. It produced effects at a concentration of ~0.01 mg/ml in the WEC. Organ systems affected were brain and facial structures whereas the heart was unaffected.	We predict that chemical 2 will affect female fertility at a comparatively low dose *in vivo* and it is also expected to cause embryotoxicity.
3	This water-soluble chemical has a low cytotoxic potency. It is positive in the EST and WEC and ReProGlo but only at a rather high concentration.	The compound may cause embryotoxic effects *in vivo* with a low potency.

- **Methyl acetoacetate**
- **Glufosinate ammonium**
- **Methoxyacetic acid**

Predictions from the Feasibility Study

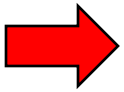
9	This lipophilic chemical has anti-androgenic activity that is detected both in the AR-CALUX and in the PALM assay. The compound is negative in the ARBA receptor binding assay suggesting the compound needs metabolic activation to act as an anti-androgen. Chemical 9 is unsuspicious in the female fertility assays. Chemical 9 is negative in the WEC and the ReProGlo assay and has a low potency in the EST. The effective concentration in this assay is close to solubility limits.	Chemical 9 is expected to affect male reproductive function and development. Furthermore. it may be a low potency embryotoxicant. ➢ **Vinclozoline**



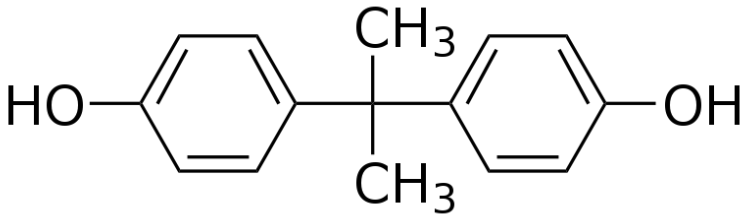
Chemical	Female fertility	Male fertility	Developmental toxicity

But:

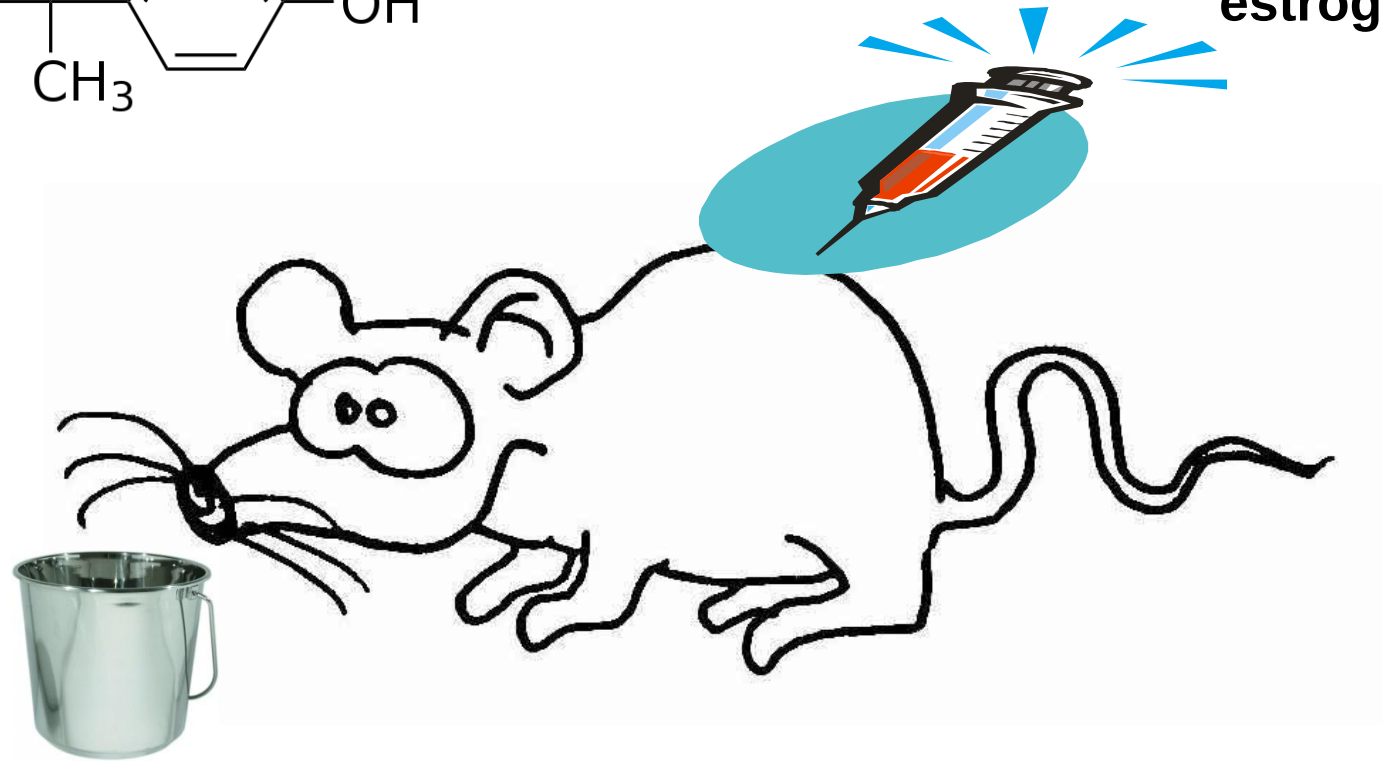
- Test chemicals mostly „black or white“ type while „grey“ might prevail in e.g. REACH chemicals
- No drugs included with a very specific MOA not easily predictable by an *in vitro* assay
- No chemicals included requiring metabolic activation through e.g. Cyp450s



Estrogenic Effects of Bisphenol A



s.c. injection:
estrogenic



oral:
first pass effect
not estrogenic

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Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

The ReProTect Feasibility Study, a novel comprehensive *in vitro* approach to detect reproductive toxicants

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[b] *German Cancer Research Center, Department of Biostatistics, Im Neuenheimer Feld 280, Heidelberg, Germany*
[c] *Institute for Health and Consumer Protection, In Vitro Methods Unit/ECVAM, Joint Research Centre, Ispra, Italy*
[d] *BioDetection Systems B.V., Science Park 406, 1098XH, Amsterdam, The Netherlands*
[e] *EggCentris NV, Z1 researchpark 310, 1731 Zellik, Belgium*
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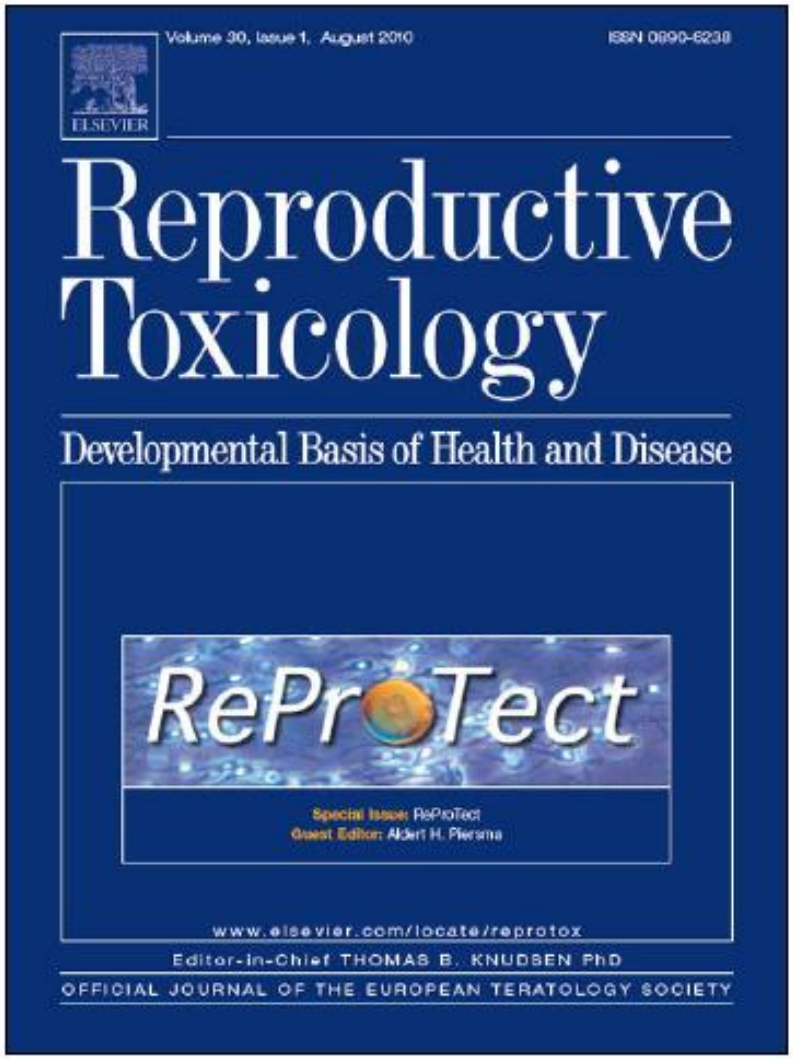


Alternatives in Reproductive Tox. testing: Present use

- **Early drug development**

  ("in-house" use for prioritization during lead compound optimization)

  Selection of candidate compounds for further safety evaluation studies; early screen-out of compounds predicted to show undesirable reproductive toxicity properties

- **Early drug development and regulatory decision making**

  Mode of action analysis for compounds that have demonstrated reproductive toxicity *in vivo*.

Alternatives in Reproductive Tox. testing:

Regulatory acceptance?

Alternatives in Reproductive Tox. testing: Regulatory acceptance?

- **REACH:**
  Use in combination with other information in a weight of evidence approach

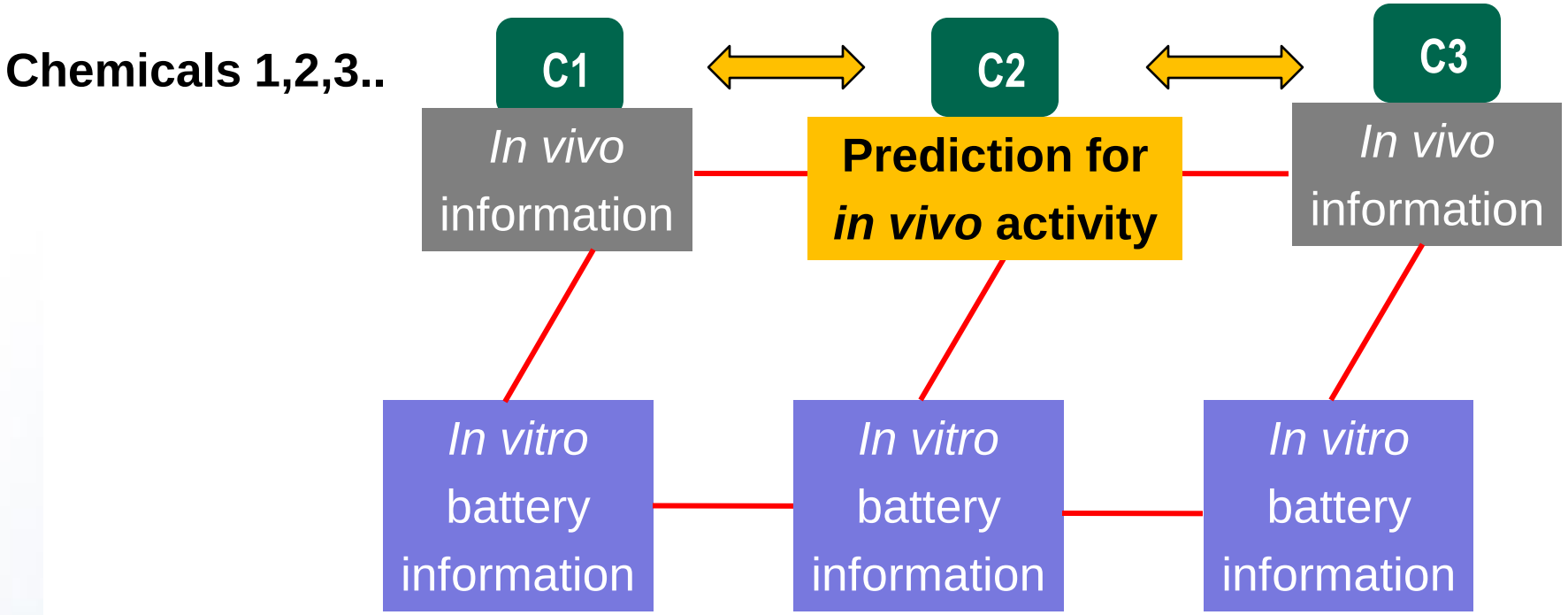
  e.g., use for justification of read across if there is doubt on the validity of the read across

http://echa.europa.eu/doc/press/webinars/read_across_and_categories_tatiana_netzeva_echa.pdf

Battery Approach in Reproductive Tox. Testing : Potential use

Chemicals (REACH):

"Parallelogram approach" for chemicals in a chemical category



Battery Approach in Reproductive Tox. Testing : Potential future use

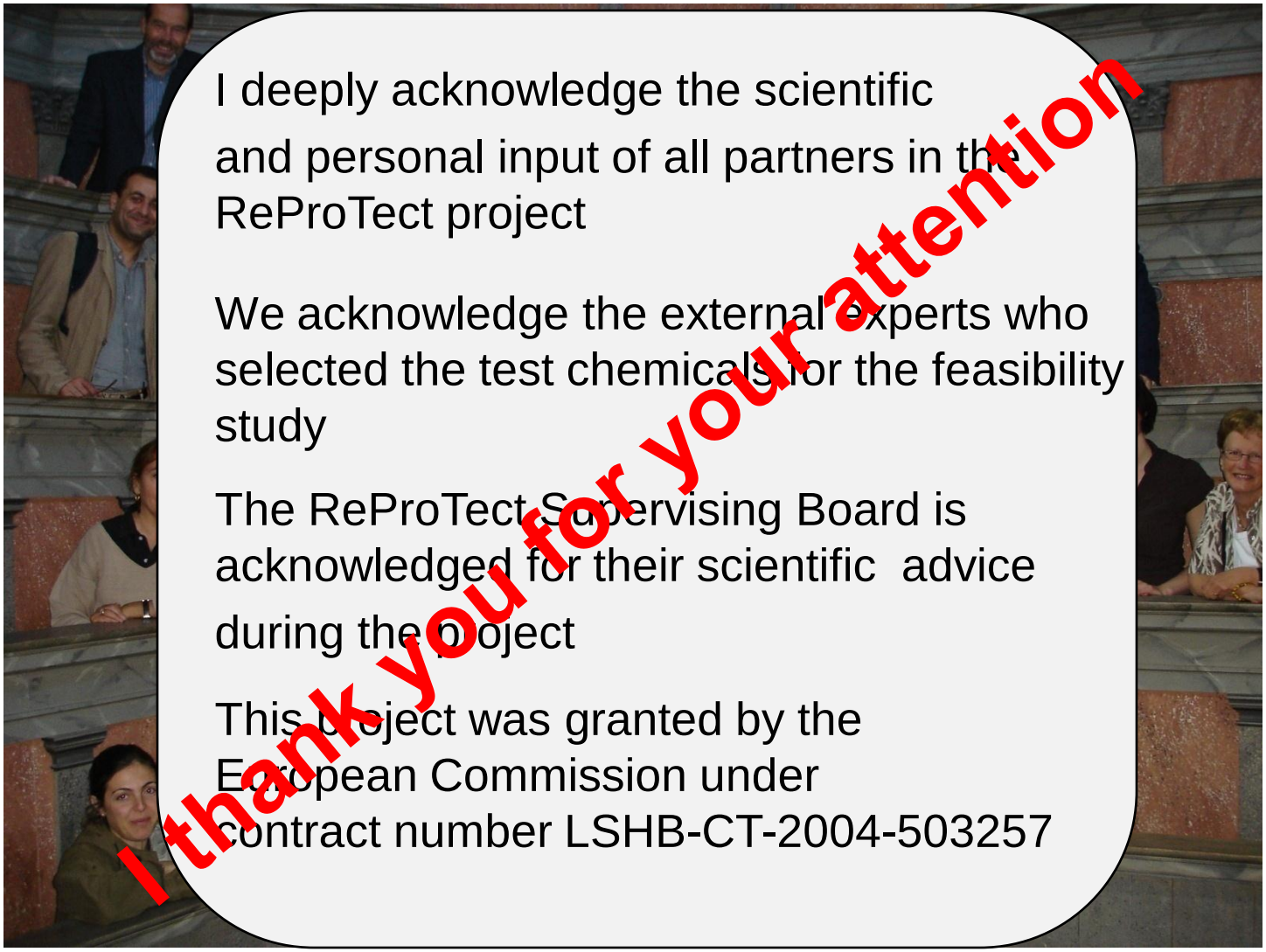
Chemical

Problem:

- **Industry** may not accept a **positive** ***in vitro*** result because of **false-positives**
- **Regulators** may not accept a **negative** ***in vitro*** result because of **false-negatives**

Acknowledgements

Uppsala 2008



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I thank you for your attention

Grouping of substances

ECHA European Chemicals Agency

Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a **group**, or "**category**" of substances.

Similarities may be based on:

- common functional group
- common precursor or break-down products
- a constant pattern in changing of potency
- common constituents or chemical classes

http://echa.europa.eu/doc/press/webinars/read_across_and_categories_tatiana_netzeva_echa.pdf

Chemical categories

ECHA European Chemicals Agency

- Common behaviour or consistent trends are generally associated with a common mechanism/mode of action
- The use of a category approach provides a basis on which to identify possible trends in properties across the category
- A substance can belong to more than one category
- Category would ideally include all potential members when first developed