Developing regulatory recognised in vitro methods (why, to whom, what, how) and the PARERE process

Tuula Heinonen
Finland´s PARERE person

WHY?

Relevance of information from animal tests to human is less than tossing a coin!

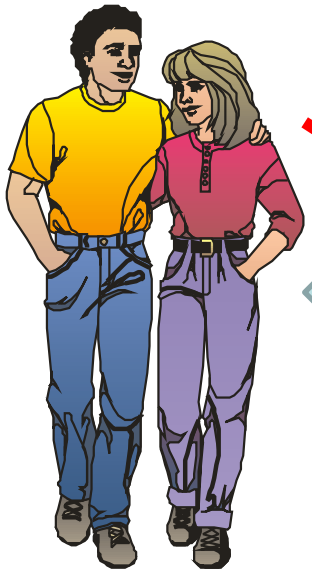
- Humans and animals have biological and physiological similarities but often differences at the molecular and cellular levels

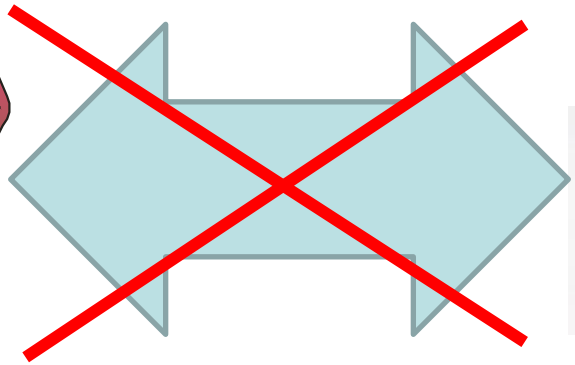
- These differences may result in different responses with regard to efficacy (drugs) and safety (all chemicals)

Finnish Centre for Alternative Methods

WHY?

Animal biology mimics poorly human biology





WHY?

Concordance of toxicity in humans and in animals is poor

- True positive average human toxicity concordance rate of pharmaceuticals was shown to be **71%** for rodents and non-rodents; with non-rodents alone being predictive for **63%** of HT and rodents alone for **43%** (Reg Tox Pharm 32, 56-67, 2000):

- Around **50%** of all long-term used pharmaceuticals induce rodent (rat or mouse) tumors

WHY?

Concordance of the teratogenicity in humans and in animals is poor

- Concordance of the teratogenicity between mice, hamster, monkey is **57%**.
- Predictive values of **60%** and **54%** for human teratogens and human non-teratogens, respectively, have been detected (Basketter et al.: A roadmap for the development of alternative (non-animal) methods for systemic toxicity testing-t3 report Altex 29(1); 3-91, 2012)

- Fewer than **one** in **40** of the substances designated as potential teratogens from animal studies, were conclusively linked to human birth defects (Knight: Systematic Reviews of Animal Experiments Demonstrate Poor Human Clinical and Toxicological UtilityATLA **35**, 641–659, 2007)

WHY?

Animal biology mimics poorly human biology: T2DM example

- Type 2 diabetes mellitus (T2DM) is globally growing health concern.
- Rodent models are widely used to develop pharmaceuticals for T2DM but without success.
- Referring to the article of Charukeshi and Pippin rodents differ at every level of glucose regulation, from
  - gene/protein expression
  - cellular signaling
  - tissue and organ to whole organism level

  when compared to that of human being

- Therefore, rodents were/are not relevant to model human T2DM. They mimic the T2DM in rodents.

Charukeshi and Pippin, Altex, 31(2), 2014

WHY?

Humanised mouse models might not mimick the effects in man

- Humanised mouse models are used as key models in pharmaceutical development and in basic research.
- Report of Seok et al., showed that human and mouse differ in what genes are involved, in the timing and duration of gene expression
- These species-specific differences may lead to wrong scientific conclusions.
  - Humanised mouse (disease) models are increasingly used in drug development and in investigations of disease mechanism.
  - The failure rate in drug development has increased. Now being 92%.
  - 56% of failures are due to not acceptable efficacy.

(Seok et al., PNAS February 26, 2013; Arrowsmith and Miller: Nature Reviews Drug Discovery Vol.12:569, 2013)

WHY?

Pharmaceuticals are just one class of chemical products

How about the other chemical substances???

- biocides
- industrial chemicals
- food and feed additives
- food packaging material

More human predictive, cheap and automated tests

To Whom?

70 million
Chemicals synthesized:
-Industrial chemicals
-Pharmaceuticals
-Cosmetics
-Biocides
-Pesticides
-Agrochemicals
-Packaging materials

- Safe and efficient chemicals and products
- Environmental risk

- **Pharma**
- **Chemical industry**
- **Cosmetics industry**
- **Biocide industry**
- **Food and feed industry**

- Marketing license
- Registration (may lead to restriction and
- authorisation or withdrawal from the market)
- CLP

Industrial chemicals

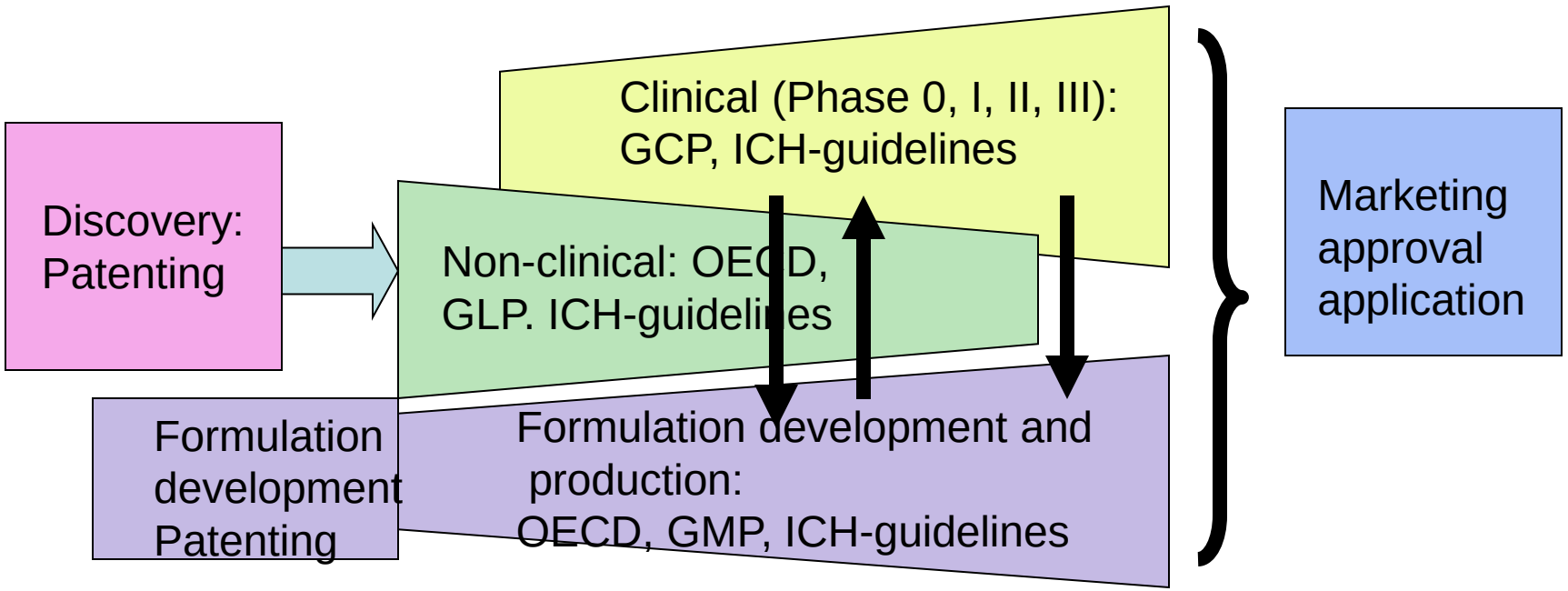
To whom

- Approximately 90 % of chemicals on the market in US and EU lack to some extent or in some cases all the required toxicity information set by regulators
- Prevalence of many diseases e.g. cancer, allergy, autism, ADHD increased

Drug development process

To Whom?

92% of new drugs fail clinical trials even though they have succesfully passed animal tests



3-5 years

5 – 10 years

1 – 2 years

Meetings with regulators: EMEA, FDA, national

Information needs for assessment of human safety

What

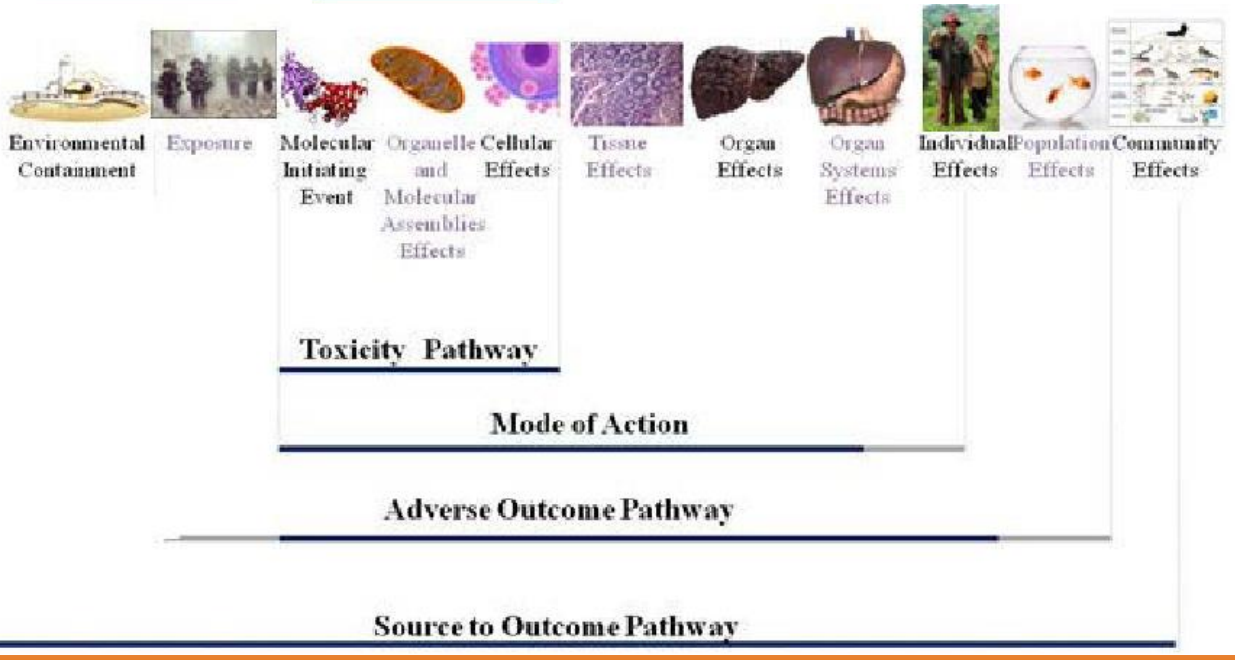
- Acute systemic toxicity
- Repeated dose systemic toxicity (2 weeks, 1 mo, 3 mo, 6 mo, 9 mo)
- Genotoxicity (gene and chromosomal levels)
- Carcinogenicity (2 years)
- Development and reproduction toxicity (several tests)
- Local tolerance (irritation and corrosion) (skin, eye, gut, lungs)
- Special toxicity: immunotoxicity, neurotoxicity
- Toxicokinetics
- ADME: absorption (skin, oral, i.m), distribution, metabolism, excretion
- For drugs: safety pharamcology

Tutkimukset tehtävä GLP:ssä (poikkeuksena jotkut ADME ja turvallisuusfarmakologiset tutkimukset)

Toxicity assessement should be performed using human cellular 2D/3D tissue/organ models and be based on AOP-principles

How

Toxicant	Macro-Molecular Interactions	Cellular Responses	Organ Responses	Organism Responses	Population Responses
Chemical Properties	Receptor/Ligand Interaction DNA Binding Protein Oxidation	Gene activation Protein production Altered signaling	Altered physiology Disrupted homeostasis Altered tissue development/ function	Lethality Impaired Development Impaired Reproduction	Structure Extinction



OECD GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS , ENV/JM/MONO(2013)6, No. 184

In vitro tests

How

- Human cell based tissue/organ models
- Measured as effects on key AOPs and there key events
- Unlimited number of concentrations (doses) can be tested
- Systemic effect obtained from modular testing – man-on-a-chip

Method development and validation process at FICAM

How

- Research model: scientifically solid model. Deliverable: publication

↓

- Optimised model: all conditions optimised, method tested with the chemical substances it will be used by end users. Deliverable: SOP

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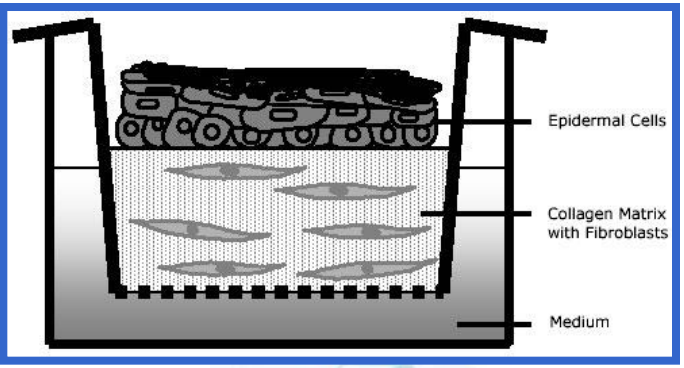
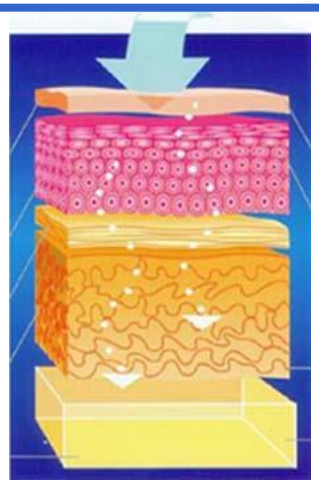
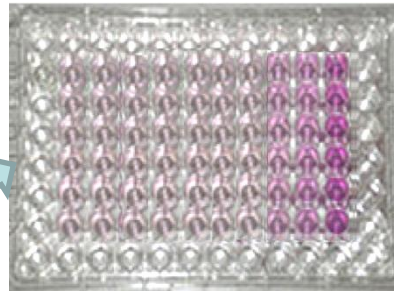
- Validated model: validation according to relevant quidelines. Deliverable: intra-laboratory validated method.

In Vitro phototoxicity in skin- Reconstructed Human Epidermis Test Method



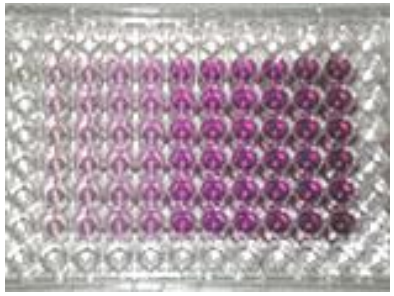


Set 1 (+UV/VIS): Irradiate with full spectrum solar light



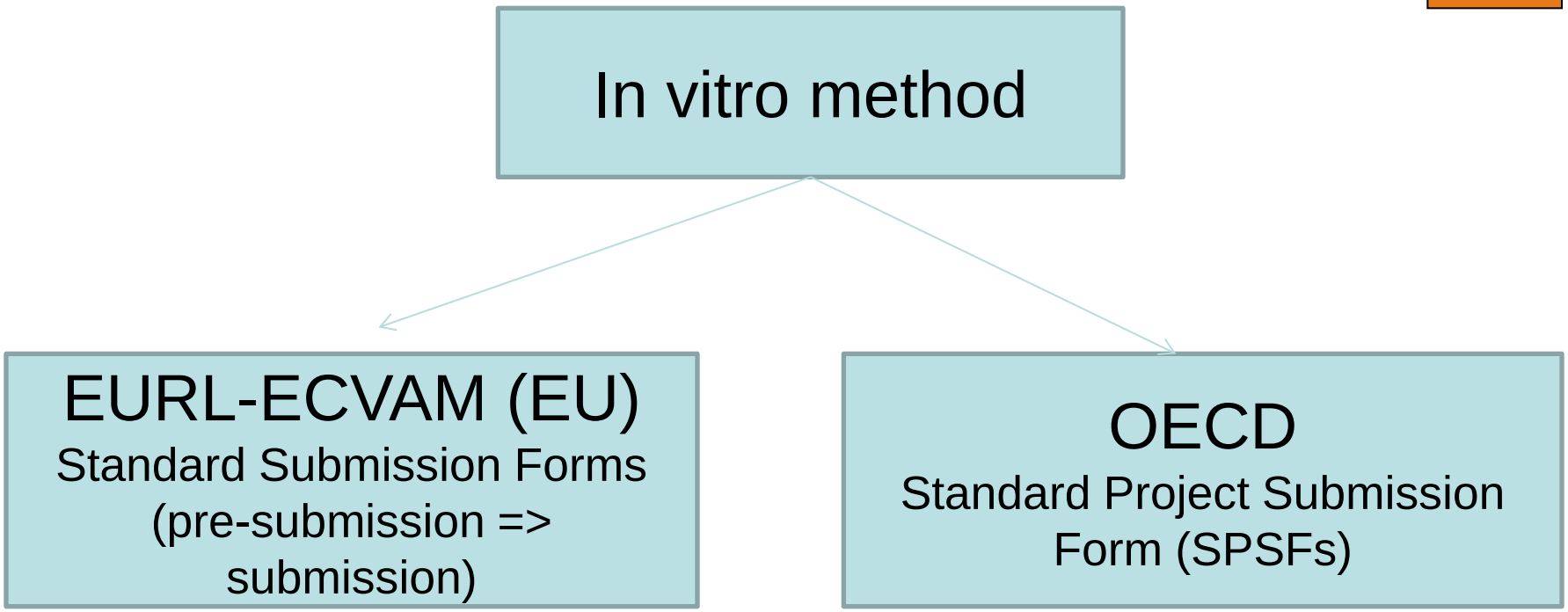
Prediction Models:
Decrease in viability (MTT-assay) > 25-30% in any of tested concentrations = Phototoxic. Additional parameters can be screened: IL-1, IL-8, Histology, protein content

Set 2 (-UV/VIS): Kept in dark



How to get new methods to be accepted by regulatory authorities?

How



What is ECVAM (EURL ECVAM)

- European Union Reference Laboratory on Alternatives to Animal Testing as specified in the **New Directive 2010/63/EU**. on the protection of animals used for scientific purposes
- Tasks:
  - Develop alternative tests to animal experiments
  - Prevalidate developed tests of own or others
  - Coordinate validations at European level
  - Database Services
  - Communication: Organise EURL ECVAM Workshops & Task Forces. **One task force is PARERE-Network.**
  - Provide scientific and technical advice to Commission services, such as to DG Environment, DG Enterprise, DG Health and Consumer Protection, DG Research, and to undertake projects with relevance to validation activities.

What is PARERE?

- On Directive 2010/63/EU on the protection of animals used for scientific purposes
- PARERE=Preliminary Assessment of Regulatory Relevance.
- Member States shall appoint a single point of contact i.e. PARERE person.
- PARERE consist of persons
  - One from each member countries (Single point of contact)
  - Representatives of EURL ECVAM
  - Representatives from Union Agencies ECHA, EMA, EFSA
- Assessing the **regulatory relevance** of submitted new tests to EURL ECVAM i.e. helping EURL ECVAM to decide whether the submitted test is needed in the first place and to priorities the submitted tests for ECVAM-validation

What are PARERE person doing in practice?

- Each PARERE person should create a network of regulatory experts from different areas in their own country.
- This network then evaluates and prioritises the submitted tests received from EURL ECVAM.
- The evaluation and prioritisation results (documents) are send to EURL ECVAM by e-mail.
- One meeting per year in EURL ECVAM. Each PARERE person should participate.
- PARERE persons should also promote alternative methods in their member countries (awareness, advices of ECVAM process, helping methods to enter validations)

EU-PARERE network: 45 persons

Patric	AMCOFF
J. Gabriel	BEECHINOR
Susanne	BELZ
Zuzana	BIROSOVA
Anne	BRAUN
Emilie	BRISORGUEIL
Heidi	BUGGE
Emma	CAREY
Petra	CEBASEK
Sandra	COECKE
Raffaella	CORVI
Emma	DI CONSIGLIO
OSCAR	DIGNOES
Huguette	DÉCHARIAUX
Federal Institute	FOR RISK ASSESSMENT
Claudius Benedict	GRIESINGER
Betty	HAKKERT
Krisztián	HARSÁNYI
Tuula	HEINONEN
Susanne	HOEKE
Marcelle	HOLLOWAY
Miriam	JACOBS
Nicholas	JARRETT
Kristina	KEJLOVA
Karin	KILIAN
AGLAIA	KOUTSODIMOU
PILAR	LEON
Kimmo	LOUEKARI
Satu Susanna	LOUHIMIES
Ana	MARTINS
Daniela	MAURICI
Birgit	MERTENS
Martin	PAPARELLA
Maria Del Pilar	PRIETO PERAITA
Elisa	REGENT
Vicky	ROBINSON
Teodora	SARAKOSTOVA
Mats	SJÖQUIST
Peter hammer	SORENSEN
Maciej	STEPNIK
Rob	VANDEBRIEL
Tuula Anneli	VESALA
Brigitte	WESTRITSCHNIG
Maurice	WHELAN
Valerie	ZUANG

Finland´s PARERE network

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- Olavi Pelkonen (olavi.pelkonen@oulu.fi)

If you have a new routine test method

- Consider it to be sent to EURL ECVAM
- It should be a routine methods to replace or supplement animal experiment in the regulatory context
- If you need any help, you may ask from Finland´s PARERE person

Ficam
Finnish Centre for Alternative Methods

KIITOS!

mmm.fi
MINISTRY OF AGRICULTURE AND FORESTRY

PIRKANMAA HOSPITAL DISTRICT
Together for better health

Leverage from the EU 2007–2013

European Union
European Regional Development Fund

UNIVERSITY OF TAMPERE

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