Alternativer til dyreforsøg - de tre R'er

Med hovedvægt på - REPLACEMENT

Min baggrund er cand.scient. i biokemi og PhD i biomedicin. Eksperimentel erfaring med dyreforsøg og in vitro metoder med anvendelse af celler fra mennesker og dyr.

Dansk repræsentant i ESAC, følgegruppe til EU's Forskningscenter vedr. alternativer til dyreforsøg.

Koordinator af EU-projekt, som bl.a. etablerer moderkage system til undersøgelse af transport af stoffer fra moder til foster.

Københavns Universitet
Miljømedicin

Dyreforsøg giver os viden om stoffers virkning i hele organismer

til forskel fra isolerede celler eller organer om

Akut toksicitet

Irritation

Langtidseffekter - kræft, mutagen, reproduktion

Dyreforsøg anvendes i dag ved

Testning af nye kemikalier på markedet
Testning af eksisterende kemikalier på markedet

Udvikling af lægemidler m.m.
Testning af lægemidler
Testning af andre forbrugerprodukter

Områder hvor vores viden er utilstrækkelig - feks forskelle i følsomhed mellem børn og voksne

What is an alternative method?

Three types of alternative methods:

- ***Reduction alternatives*** obtain a comparable level of information from the use of fewer animals, or more information from the same number of animals
- ***Refinement alternatives*** minimise pain, suffering and distress
- ***Replacement alternatives*** permit a given purpose to be achieved without using animals

Reference:

Russell, W.M.S. & Burch, R.L. (1959). The Principles of Humane Experimental technique. Methuen, London.

Hvilke alternativer findes inden for ’replacement’

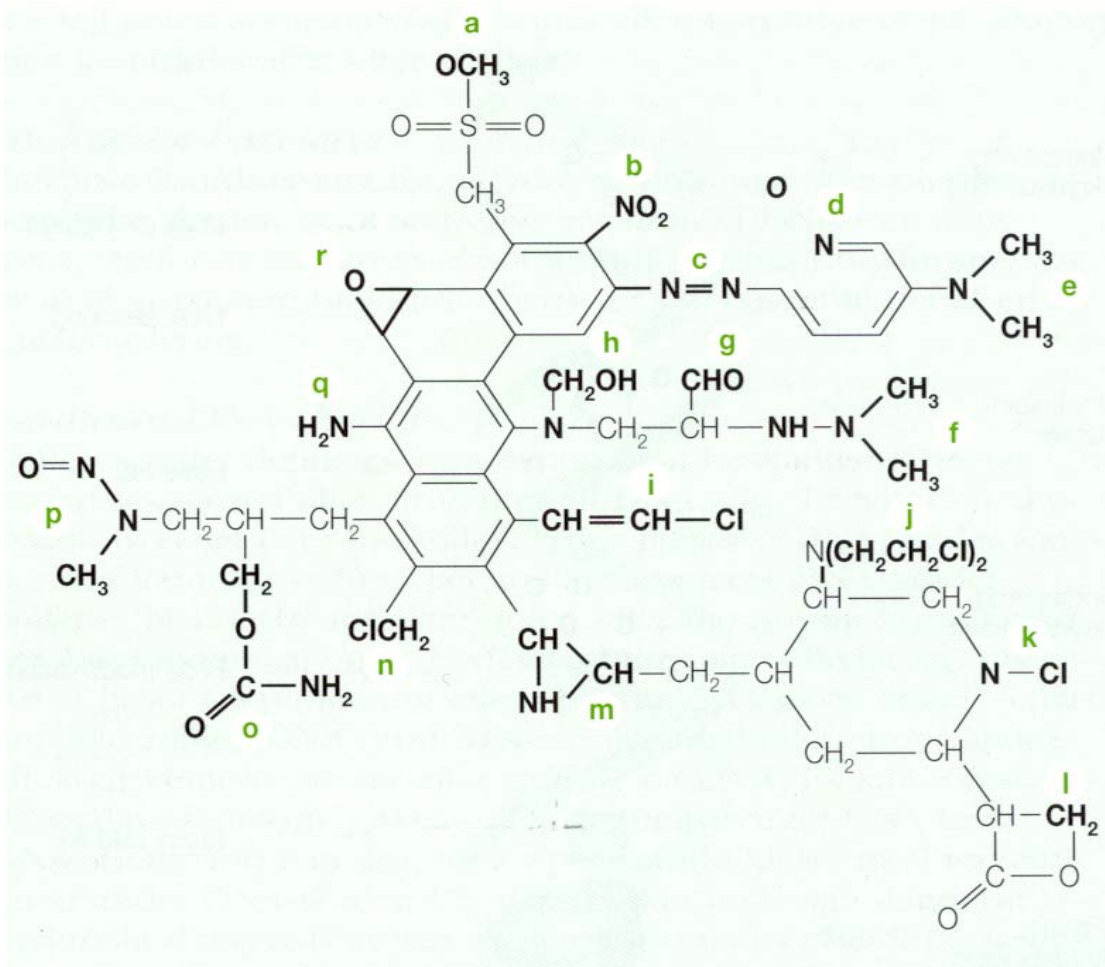
Struktur analogi overvejelser

Analogi med beslægtede stoffer

Teste på menneske (eller dyre) celler

Teste på forsøgspersoner, evt patienter

Væsentlige strukturelle enheder, der anses for 'mistænkelige' strukturer i forhold til genotoksisk effekt
Ashby, 1991



Eksempel på replacement:

Erstatning af brug af kaniner med cellekulturer
til testning af vacciner for tilstedeværelse af urenheder

Kaninen (og mennesker) vil reagere med temperatur
stigninger, hvis der er urenheder i form af pyrogener tilstede

Cellekulturen vil reagere med udskillelse af målbare
komponenter, hvis der er urenheder til stede

Eksempel på replacement:

RANTIV: Risk Assessment for Neurotoxins Tested In Vitro

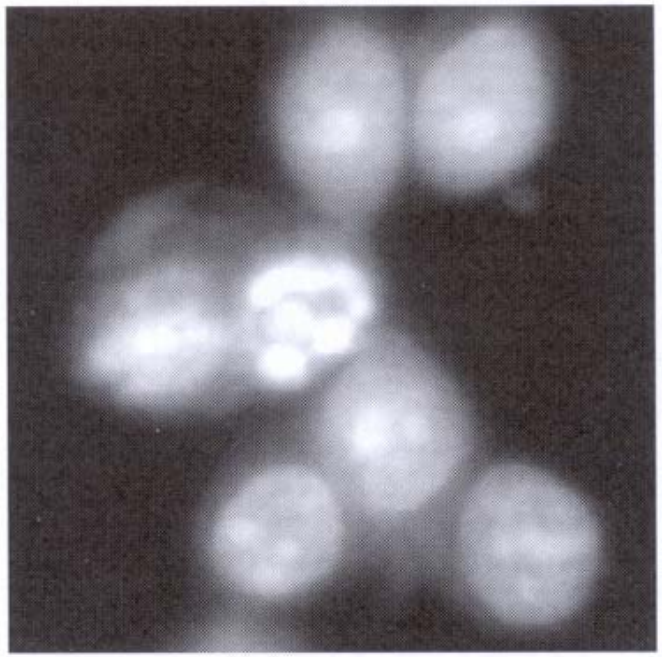


Fig 2. Nuclear fragmentation in neuronal cells exposed to a metabolite of the toxic volatile compound styrene

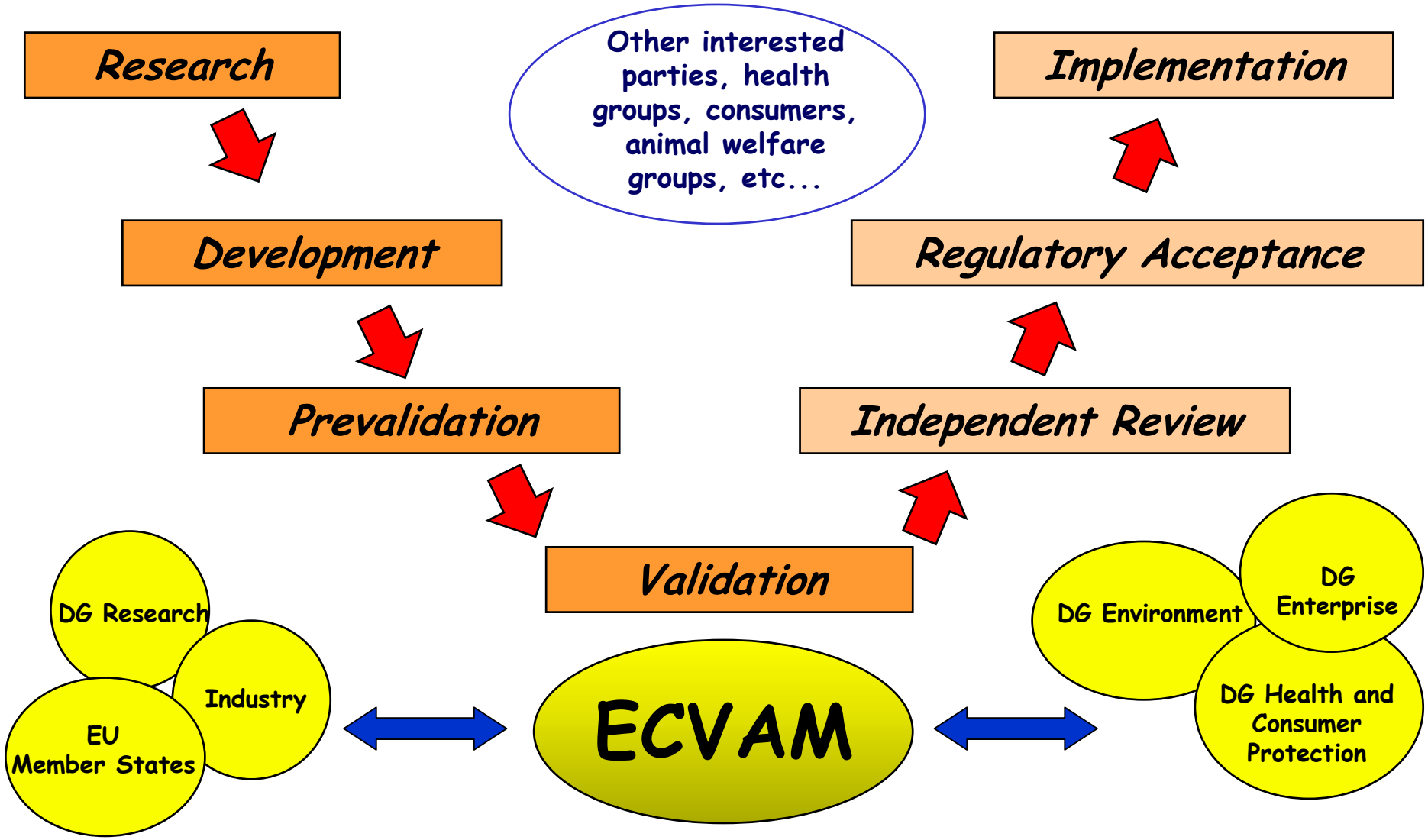
Hvorfor går det så langsomt med udviklingen af alternativer til dyreforsøg

Validering tager tid

Der er ikke stor anerkendelse af alternative i den videnskabelige verden

Der mangler incitamenter i form af styring fra bevillingsgivere

ECVAM AND PROTECTION OF HEALTH OF THE CITIZEN



What does it mean to "validate" a method?

… to establish the reliability and relevance of the method for a particular purpose

Reliability: reproducibility of results within and between laboratories and over time

Relevance: scientific value and practical usefulness

Purpose: the intended application of the procedure

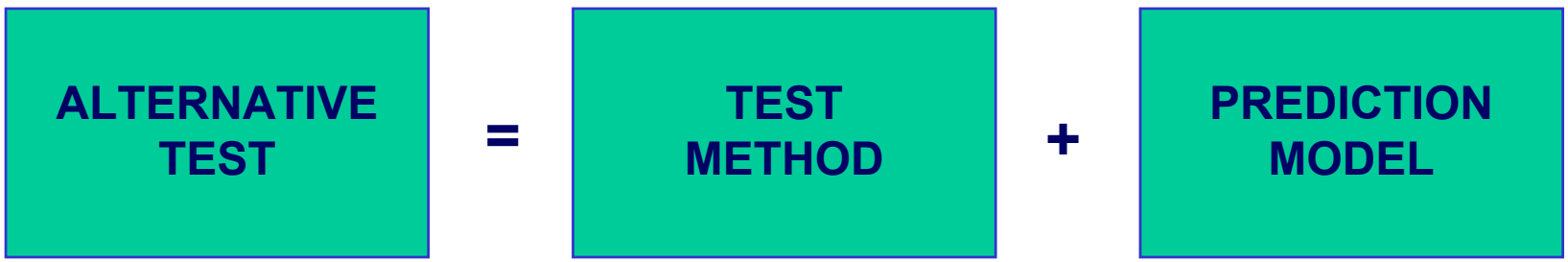
This definition applies to both alternative AND animal methods

References:
(1) The Amden CAAT/ERGATT workshop (ATLA 18, 313-337, 1990)
(2) Frazier's report to the OECD (OECD Environment Monograph no. 36)

What is an alternative (replacement) test?

An alternative test can be regarded as the combination of a test system and a prediction model.

Reference: Archer et al. (1997). ATLA 25, 505-516



A prediction model (PM) is an explicit decision-making rule for converting the results of one or more alternative tests into a prediction of an *in vivo* endpoint.

Stages in the evolution of regulatory tests

1	Research and development	Supporting role of ECVAM
2	Prevalidation	Leading role of ECVAM
3	Validation	
4	Independent assessment	Scientific peer review, ESAC
5	Regulatory acceptance	Responsibility of other bodies (EU Competent Authorities)

Timescale: Prevalidation → acceptance **typically 6 years**

Criteria for test development

For entry into (pre)validation, a test should be accompanied by:

- A definition of its scientific purpose and proposed practical application
- A description of its scientific basis
- The case for its relevance, including its advantages compared with other tests
- An optimised protocol, including:
  - standard operation procedures
  - a specification of endpoints, endpoint measurement, derivation and expression of results, and their interpretation, via a prediction model
  - the inclusion of adequate controls
- A statement about limitations/domain of applicability
- Evidence of reproducibility (within labs, and if available, between labs)

Reference: Balls & Fentem (1999). Toxicology in Vitro 13, 837-846.

Post-validation

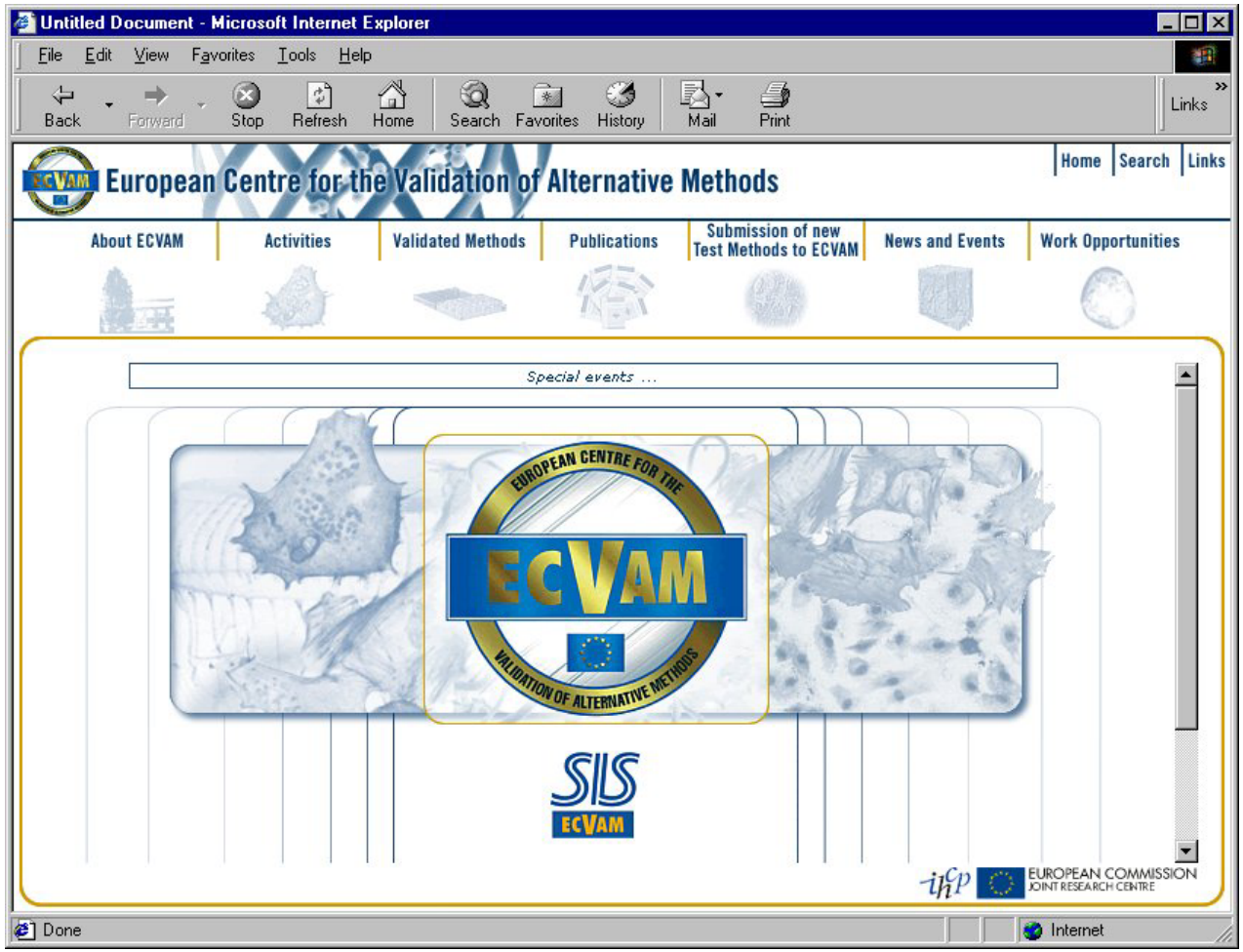
Independent assessment

- Publication of the study in a peer-reviewed journal
- Assessment of the outcome by an independent expert panel
- Statement on the validity

Regulatory acceptance

- Draft test guideline
- Submission to regulatory body
- Consultation with expert groups
- Adoption and publication of the new test guideline

http://ecvam.jrc.it



THE ECVAM SCIENTIFIC ADVISORY COMMITTEE (ESAC)

- The ESAC was established by the Commission Communication of October 1991.
- The ESAC advises on ECVAM's work programme, and formally considers the scientific validity of alternative tests.
- The ESAC members are selected representatives of the Member States, of the European chemical, cosmetic and pharmaceutical industry associations, academic toxicology and animal welfare groups, together with representatives of ECVAM's principal customer DGs.
- The ESAC meets twice each year, at Ispra.

METHODS/STATEMENTS ENDORSED BY THE ESAC - Chemicals

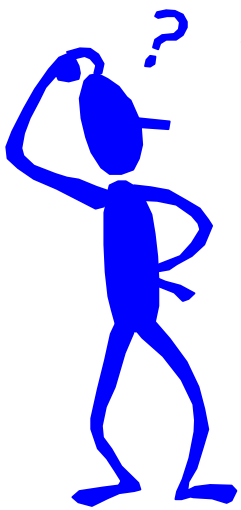
• 3T3 NRU phototoxicity test	November 1997
• EPISKIN skin corrosivity test	April 1998
• Rat TER skin corrosivity test	April 1998
• Application of the 3T3 NRU phototoxicity test to UV filter chemicals	May 1998
• Local lymph node assay for skin sensitisation	March 2000
• EpiDerm skin corrosivity test	March 2000
• CORROSITEX skin corrosivity test	December 2000
• Embryonic stem cell test for embryotoxicity	May 2002
• Whole-embryo culture test for embryotoxicity	May 2002
• Micromass test for embryotoxicity	May 2002

Accepted and incorporated into Annex V and OECD guidelines

METHODS/STATEMENTS ENDORSED BY THE ESAC - Biologicals

• In vitro production of monoclonal antibodies	May 1998
• ELISA test for batch potency testing of tetanus vaccines for human use	December 2000
• ToBI test for batch potency testing of tetanus vaccines for human use	December 2000
• ELISA test for batch potency testing of erysipelas vaccines	June 2002
• Relevance of the target animal safety test for batch safety testing of vaccines for veterinary use	June 2002
• Deletion of a test in polycythaemic mice for batch potency testing of erythropoietin concentrated solution	June 2002

Accepted and incorporated into European Pharmacopoeia monographs



WHAT ?

ecopa

European Consensus Platform on Alternatives

- **International Not-For-Profit Organisation in Belgium (Europe)**
- **Members : National Consensus Platforms on Alternatives with 4 parties :**

Academia | **Government**

Industry | **Animal Welfare**

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

EU WHITE PAPER STRATEGY FOR A FUTURE CHEMICALS POLICY

EINECS
100.000 "old" substances

ELINCS
3000 NEW substances

> 95% ???

REACH
Registration, Evaluation, Autorisation, with PRIORITIES
30.000 chemicals

9.6 – 12.8 million animals

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

EU WHITE PAPER STRATEGY FOR A FUTURE CHEMICALS POLICY

HIGH NEED FOR 3R-ALTERNATIVES

⇑

9.6 – 12.8 million animals

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

7th AMENDMENT OF EU COSMETICS LEGISLATION

⇓

ANIMAL TESTING BAN

⇓

MARKETING BAN OF COSMETIC INGREDIENTS & FINAL PRODUCTS TESTED ON ANIMALS

⇓

HIGH NEED FOR 3R-ALTERNATIVES

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

ECVAM

⇓

STRATEGIC PLAN FOR FUTURE DEVELOPMENT AND VALIDATION OF ALTERNATIVE METHODS

⇓

WITHIN NEXT 5 YRS ALTERNATIVES FOR MOST ENDPOINTS

⇓

HIGH NEED FOR 3R-ALTERNATIVES

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

WHAT 3R-ALTERNATIVES DO WE ACTUALLY HAVE IN THE EU FOR REGULATORY TESTING ?

4 VALIDATED METHODS → **ANNEX V**

Dangerous Substances Legislation

67/548/EEC

- **3 corrosivity tests**
  TER, Epi Skin®,Epiderm®
- **1 phototoxicity test**
  3T3 NRU-PT

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

WHAT 3R-ALTERNATIVES DO WE ACTUALLY HAVE IN THE EU FOR REGULATORY TESTING ?

4 VALIDATED METHODS

6 METHODS ACCEPTED BY ESAC

NOT YET TAKEN UP IN EU LEGISLATION

- 1 skin sensitisation test: LLNA
- 1 *in vitro* percutaneous absorption test
- 1 additional corrosivity test: Corrositex®
- 3 embryotoxicity tests: WEC, MM, ECT

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

WHAT 3R-ALTERNATIVES DO WE ACTUALLY HAVE IN THE EU FOR REGULATORY TESTING ?

4 VALIDATED METHODS

6 METHODS ACCEPTED BY ESAC

TESTS UNDER DEVELOPMENT & VALIDATION

- skin irritation
- eye irritation
- acute (oral) toxicity
- skin corrosion (in silico)

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

WHAT 3R-ALTERNATIVES DO WE ACTUALLY HAVE IN THE EU FOR REGULATORY TESTING ?

- 4 VALIDATED METHODS
- *IN VITRO* GENOTOXICITY TESTS
- 6 METHODS ACCEPTED BY ESAC
- TESTS UNDER DEVELOPMENT & VALIDATION

POSITION TOWARDS ACTUAL EU POLICY AND ALTERNATIVE METHODS

ALTERNATIVE TESTS NEEDED FOR :

- PHOTO ALLERGY
- SUBACUTE TOXICITY
- CHRONIC TOXICITY
- REPRODUCTIVE TOXICITY
- TARGET ORGAN & SYSTEMIC TOXICITY
- BIOKINETICS
- NON-GENOTOXIC CARCINOGENICITY

LACKING TODAY

Præsentationen er udarbejdet med bidrag fra
Marlies Halder, ECVAM

og Vera Rogiers, ECOPA

Endvidere er der anvendt billeder fra
www.imm.ki.se/EURANTIV
og Uffe Midtgård, Leif Simonsen, Lisbeth E. Knudsen:
Tksikologi i arbejdsmiljøet, bind II