# DISAPPOINTMENT OR HOPES FOR NEW METHODOLOGY ?


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# LAYOUT OF THE TALK

- CURRENT STATUS ON VALIDATED ALTERNATIVES
- AVAILABLE VALIDATED ALTERNATIVES: HOPES AND DISAPPOINTMENTS
  1. Murine Local Lymph Node Assay
  2. 3T3 Neutral Red Uptake Phototoxicity test
  3. Embryonal Stem Cell Test
  4. EPISKIN™ *in vitro* test for skin irritation testing
- EXPECTATIONS FOR THE FUTURE
- CONCLUSIONS

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# WHAT VALIDATED 3R-ALTERNATIVES DO WE ACTUALLY HAVE ?

| | |
|---|---|
| ☺ ACUTE ORAL TOXICITY | Fixed dose<br>Acute toxic class<br>Up-and-down |
| ☺ SKIN CORROSIVITY | TER, EPISKIN™, EpiDerm |
| ☺ SKIN IRRITATION | EPISKIN™ |
| ☺ SKIN SENSITISATION | LLNA (rLLNA) |
| ☺ PHOTOTOXICITY | 3T3 NRU PT |
| ☺ DERMAL ABSORPTION | *In vitro* (human / pig) |
| ☺ MUTAGENICITY | Ames<br>*In vitro* mammalian cell mutation<br>*In vitro* micronucleus<br>*In vitro* mammalian chromosome aberration |
| ☺ EMBRYOTOXICITY | WEC, MM, EST |

**ADDRESSING HAZARD IDENTIFICATION FOR ACUTE AND LOCAL TOXICITY**

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# ARE THE CURRENTLY AVAILABLE ALTERNATIVE METHODS SUITABLE FOR TESTING DIFFERENT COMPOUND CATEGORIES ?

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## ❶ Murine Local Lymph Node Assay

Basketter D., April 2007,
VUB, Brussel

## ❶ Murine Local Lymph Node Assay

**ACTUAL STATUS:**

1992: OECD screening

1999: ICCVAM approval

2000: ESAC approval

2002: OECD 429

2004: EC B.42

New developments: rLLNA (reduction)

non-radioactive LLNA

## ❶ Murine Local Lymph Node Assay

**ACTUAL STATUS:** OECD 429 (2002), 67/548/EEC Annex V - B.42 (2004)

**EXPERIENCE GAINED:**

| | Non-LLNA | Non-LLNA & LLNA | Only LLNA |
|---|---|---|---|
| CHEMICALS*<br>4573 compounds<br>in database | 1998-2007<br>3330 compounds | 1998-2007<br>56 compounds | |
| COSMETICS**<br>176 compounds<br>in database | < 2002<br>70 compounds | < 2002<br>26 compounds | |
| | ≥ 2002<br>1 compound | | ≥ 2002<br>22 compounds |

* ECB data (new chemicals database), presented by Jens Linge, *epaa* Lyon, 1-2/10/2007

** Databank compiling publicly available data SCCP opinions,
Pauwels M & Rogiers V, Vrije Universiteit Brussel

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| | ≥ 2002<br>1 compound | | ≥ 2002<br>22 compounds |

⇒ **EXAMPLE OF A WELL-DEVELOPED ALTERNATIVE**

⇒ **BUT STILL FOLLOW-UP NEEDED** (Basketter D., *epaa* Lyon, 1-2/10/2007)

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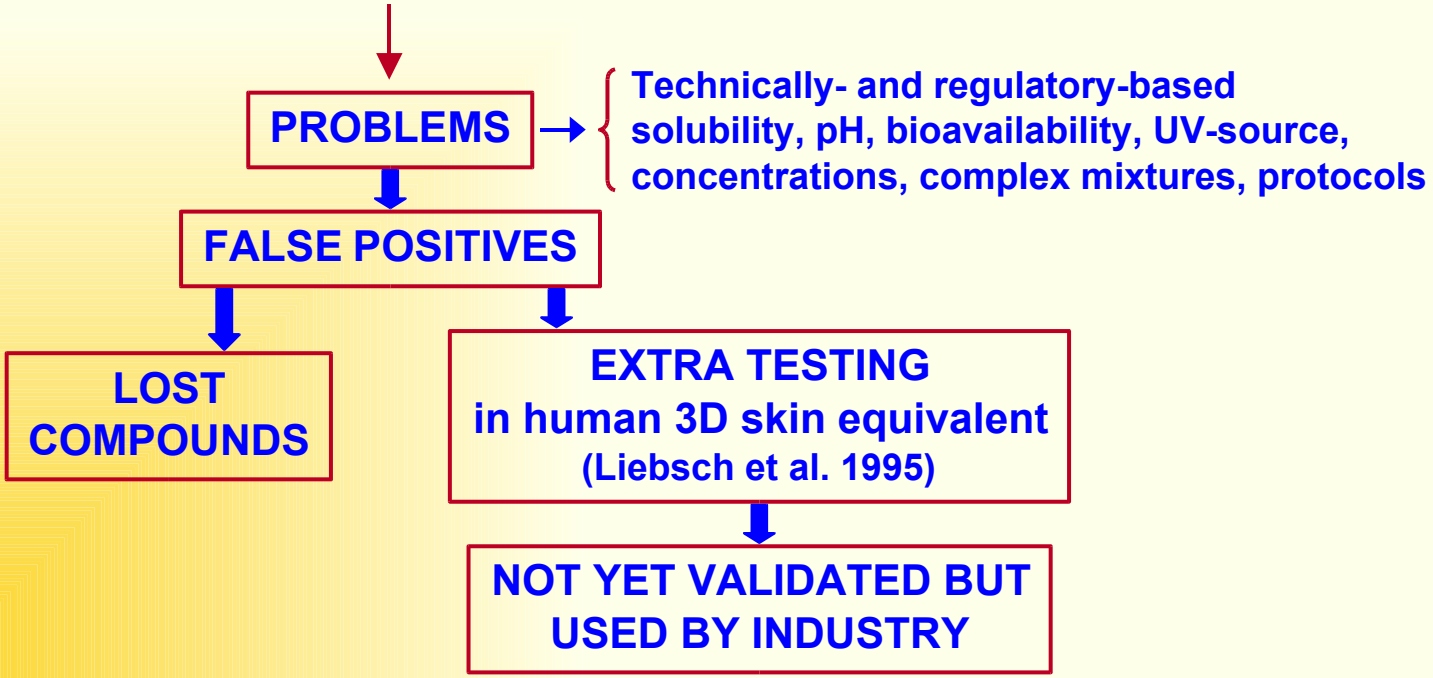
## ❷ 3T3 Neutral Red Uptake Phototoxicity test

## ❷ 3T3 Neutral Red Uptake Phototoxicity test

**ACTUAL STATUS:** in 2004: OECD 432
in 2000: EC B.41

**EXPERIENCE GAINED:**
- ☺ for chemicals
- ☺ for UV filters (cosmetics)
- ☹ for pharmaceuticals (De Smet A., J&J, *epaa*, Brussels, 5/11/2007)



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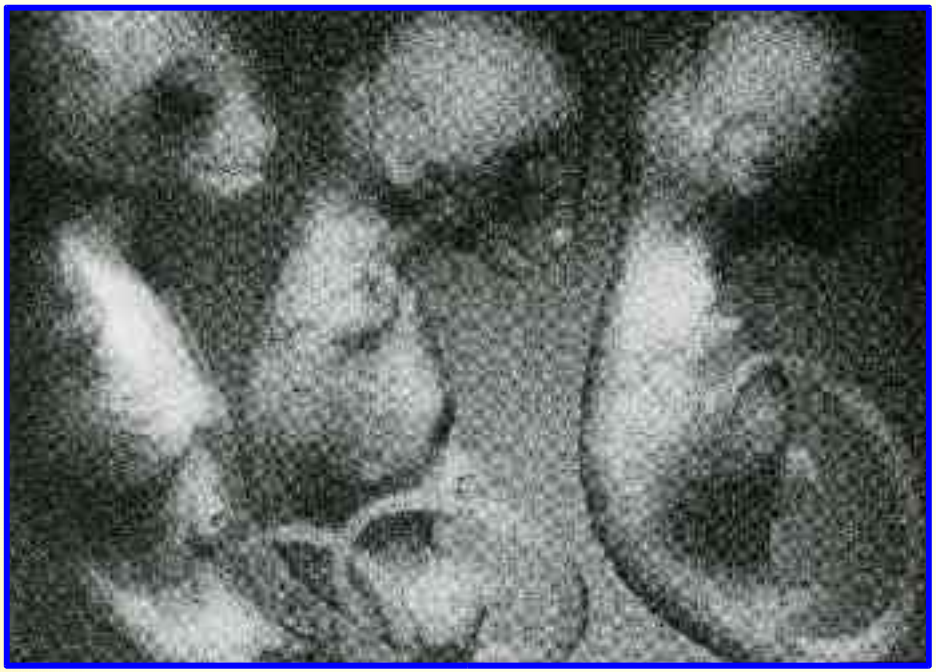
## ❸ Embryonal Stem Cell Test



Marquardt et al., Toxicology (1999)

## ❸ Embryonal Stem Cell Test

**ACTUAL STATUS:** ESAC approval (2001)
Regulatory refusal (???)

**EXPERIENCE GAINED:**
- ☺ for chemicals (Spielmann et al. ZEBET)
- ☹ for cosmetics: not accepted by SCCP
- ? for pharmaceuticals : ☺ or ☹ ??
  discussions about predictivity

⇒ **EST NOT SCIENTIFICALLY READY FOR REGULATORY ACCEPTANCE**

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## ❹ EPISKIN™ *in vitro* test for skin irritation testing


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**ACTUAL STATUS:** ESAC approval (2007) as a stand-alone with MTT reduction as endpoint (optional is IL-1α)

**EXPERIENCE GAINED:**

☺ for chemicals
- 60 chemicals on reference list
- in-house data (coded)

? for cosmetics
- only 1 chemical on Annexes 76/768/EEC
- no data on hair dyes/colourants

→ **CONCERNS ABOUT MTT-REDUCTION AS ENDPOINT AND BARRIER FUNCTION OF *IN VITRO* MODEL**

Toxicol in vitro 21, 2007

## ❹ EPISKIN™ *in vitro* test for skin irritation testing

**EXPERIENCE GAINED: ? for cosmetics**

***In vivo* skin irritation data extracted from SCC(NF)P opinions (2000-2006)***

| | *In vivo* skin irritation data available | Results |
|---|---|---|
| COSMETICS* 176 compounds in database | 112 compounds | Indecisive: 1<br>Non-irritating: 75<br>Slightly/mildly irritating: 23<br>Irritating: 11<br>Severely irritating: 1<br>Corrosive: 1 |

18 of the 112 compounds provoked discolouration of skin (hair dyes), in one case scoring became impossible due to discolouration

* Databank compiling publicly available data SCCP opinions, Pauwels M & Rogiers V, Vrije Universiteit Brussel

## ❹ EPISKIN™ *in vitro* test for skin irritation testing

**EXPERIENCE GAINED: ? for cosmetics**

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⇒ **POTENTIAL BASIS FOR INGREDIENT SELECTION FOR ADDITIONAL STUDY TO SUPPORT AVAILABLE DATA**

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# HOPES FOR THE NEAR FUTURE …

## WHAT ARE THE PROSPECTS (☺) FOR THE NEAR FUTURE ?

- ☺ *In vitro* eye irritation test
- ☺ *In vitro* non-toxicity prediction (cytotoxicity)
- ☺ *In vitro* cell transformation assay
- ☺ *In vitro* skin model for genotoxicity
- ☺ *In vitro* endocrine disrupter test

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- ☺ *In vivo* one-generation study
- ☺ *In vivo* non-radioactive LLNA

## WHICH ALTERNATIVES ARE LACKING (☹) ?

- ☹ Acute dermal toxicity
- ☹ Acute inhalation toxicity
- ☹ Photoallergy
- ☹ Subacute and subchronic toxicity
- ☹ Chronic toxicity
- ☹ Target organ and systemic toxicity
- ☹ (Non-genotoxic) carcinogenicity
- ☹ Biokinetics

⇒ LACK OF ALTERNATIVES FOR SYSTEMIC AND LONG-TERM TOXICITY TESTING

⇒ PROBLEM FOR QUANTITATIVE RISK CHARACTERISATION, IN PARTICULAR FOR COSMETICS (testing & marketing ban)

# HOPES FOR NEW METHODOLOGIES ?

## ✶ TRANSCRIPTOMICS, PROTEOMICS, …

- ➢ Valuable technologies
- ➢ Mechanistic elucidation of toxicological questions
- ➢ Problems: - price and complexity
  - cell systems and *in vitro* models not good enough
    - stability of primary cells
    - lack of biotransformation enzymes in cell lines, transformed and transfected cells
    - dedifferentiation in culture (time, medium composition)
  - NOT READY FOR VALIDATION AND REGULATORY ACCEPTANCE

## ✶ NANOTECHNOLOGY

- ➢ Progress: - in understanding new dimensions and metrology in toxicity testing
- ➢ Problems: - methods are not validated
  - NOT AVAILABLE FOR COSMETICS (2009 deadline)

# HOPES FOR NEW METHODOLOGIES ?

✶ **STEM / PROGENITOR CELL RESEARCH**

- ➢ Embryonic
- ➢ Adult

⬇

**UNLIMITED SOURCE OF FUNCTIONAL HUMAN TARGET CELLS**

→ **IN RESEARCH PHASE, NO ROUTINE DELIVERY OF CELLS !!!**

✶ **SYSTEMS BIOLOGY**

⬇

**BASIC SCIENCE TO UNDERSTAND MECHANISMS AND INTERACTIVE PATHWAYS**

→ **IN RESEARCH PHASE, NO ROUTINE DELIVERY OF CELLS !!!**

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# CONCLUSIONS

- 3R-VALIDATED ALTERNATIVE METHODS: HAZARD IDENTIFICATION OF LOCAL AND SHORT-TERM TOXICITY

  - REFINEMENT AND FOLLOW-UP ARE URGENTLY NEEDED
  - APPLICABILITY IN DIFFERENT FIELDS REMAINS AN OPEN QUESTION

- LACK OF 3R-ALTERNATIVES FOR SYSTEMIC AND LONG-TERM TOXICITY

  - PROBLEM FOR QUANTITATIVE RISK ASSESSMENT OF NEW COMPOUNDS

- BASIC RESEARCH IS NEEDED (MORE THAN EVER) TO BUILD A SOLID BASIS FOR THE MORE ‘DIFFICULT PROBLEMS’

# LESSONS TO BE LEARNED

- STOP OVERSELLING ALTERNATIVE METHODS AND RAISING OF NON-REALISTIC EXPECTATIONS
- GAIN TRUST OF REGULATORY BODIES FOR ALTERNATIVES BY FOLLOW-UP AND CORRECT REPORTING
- INCORPORATE RESULTS OF 'REAL WORLD' INTO 3R-ALTERNATIVES TO COME TO USEFUL TESTS FOR ALL TYPES OF COMPOUNDS
- FOCUS ON PRIORITISATION OF THE REAL NEEDS

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