HOW GOOD ARE VALIDATED METHODS TO PREDICT TOXICITY OF DIFFERENT TYPES OF SUBSTANCES AND PRODUCTS ?

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LAYOUT OF THE TALK

- CURRENT STATUS OF VALIDATED ALTERNATIVES
- LIMITATIONS OF AVAILABLE VALIDATED ALTERNATIVES
  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
- LACKING ALTERNATIVES
- CONCLUSIONS

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CURRENT STATUS …

WHAT VALIDATED 3R-ALTERNATIVES DO WE ACTUALLY HAVE ?

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

ADDRESSING HAZARD IDENTIFICATION FOR ACUTE AND LOCAL TOXICITY

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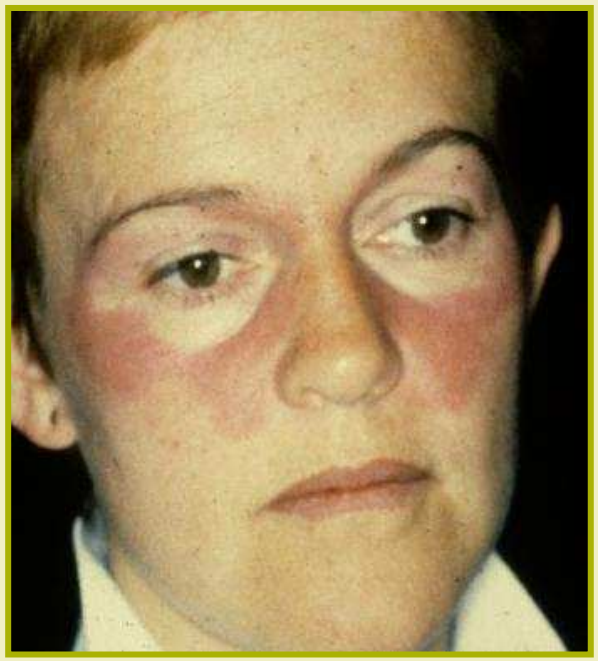
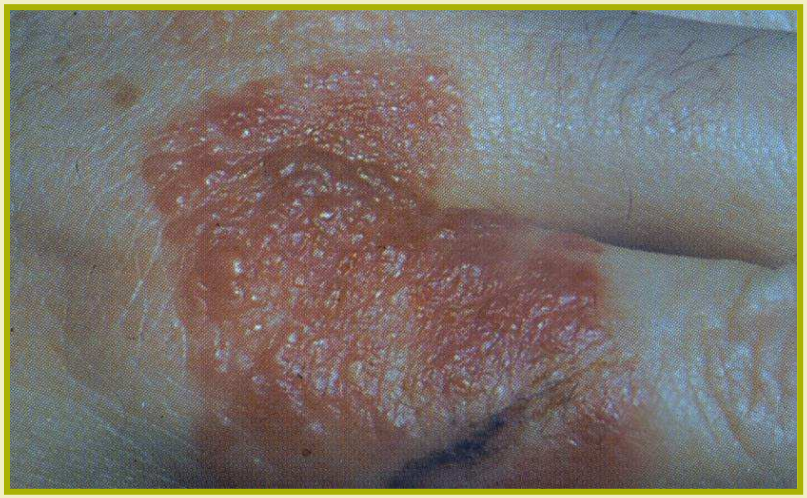
AVAILABLE VALIDATED ALTERNATIVES

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

❶ Murine Local Lymph Node Assay





Contact Allergy

Allergic Contact Dermatitis

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

CURRENTLY AVAILABLE VALIDATED ALTERNATIVES: SUITABLE FOR DIFFERENT COMPOUND CATEGORIES ?

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* 4573 compounds in database	1998-2007 3330 compounds	1998-2007 56 compounds	
COSMETICS** 176 compounds in database	< 2002 70 compounds	< 2002 26 compounds	
	≥ 2002 1 compound		≥ 2002 22 compounds

⇒ EXAMPLE OF A WELL-DEVELOPED ALTERNATIVE ?

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

* 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

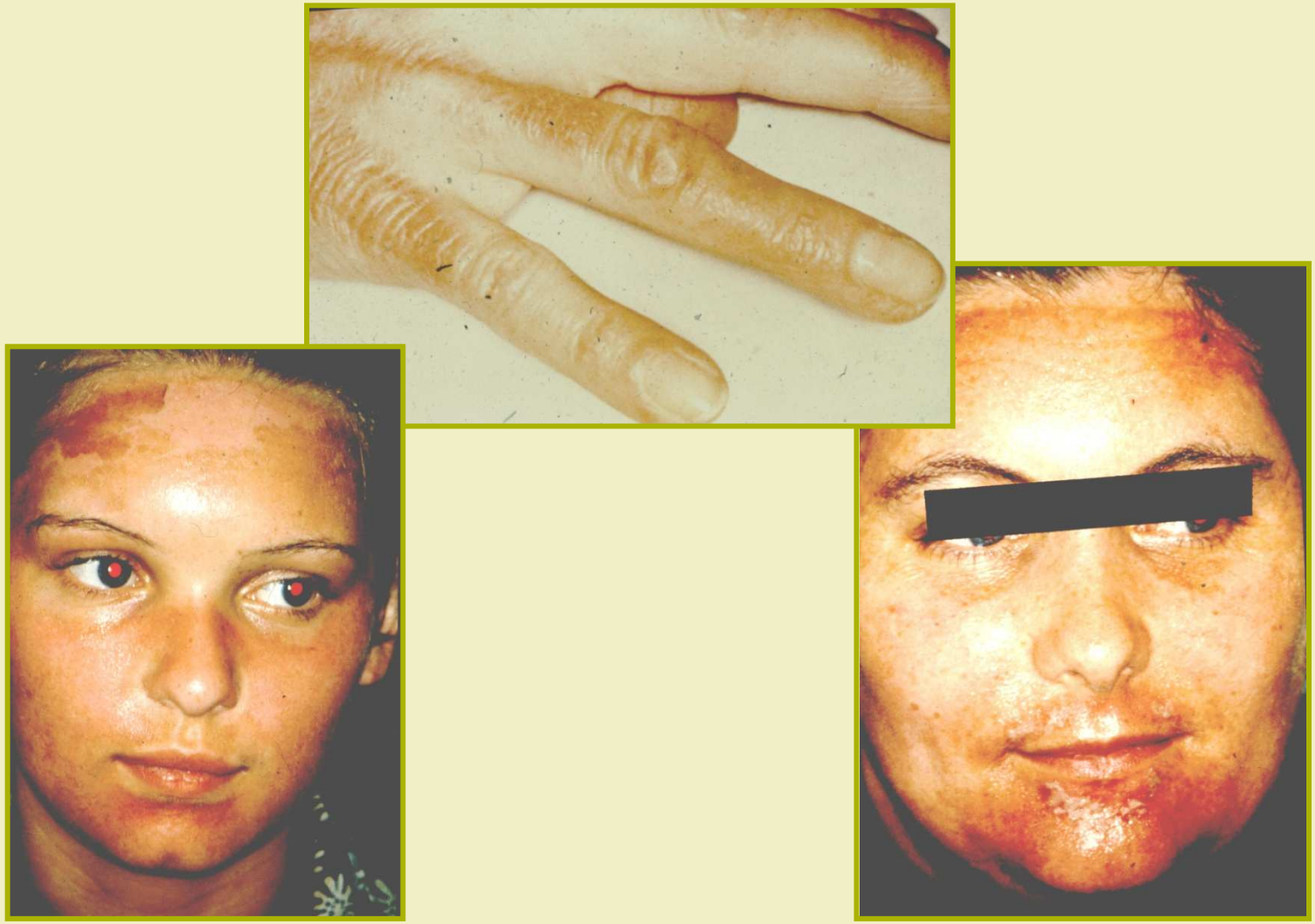
❶ Murine Local Lymph Node Assay

EXPERIENCE GAINED:

	Similar Results	Diverging Results
COSMETICS** 23 compounds in database with LLNA and MK-test	17	6 4 unreliable MK-test due to staining

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

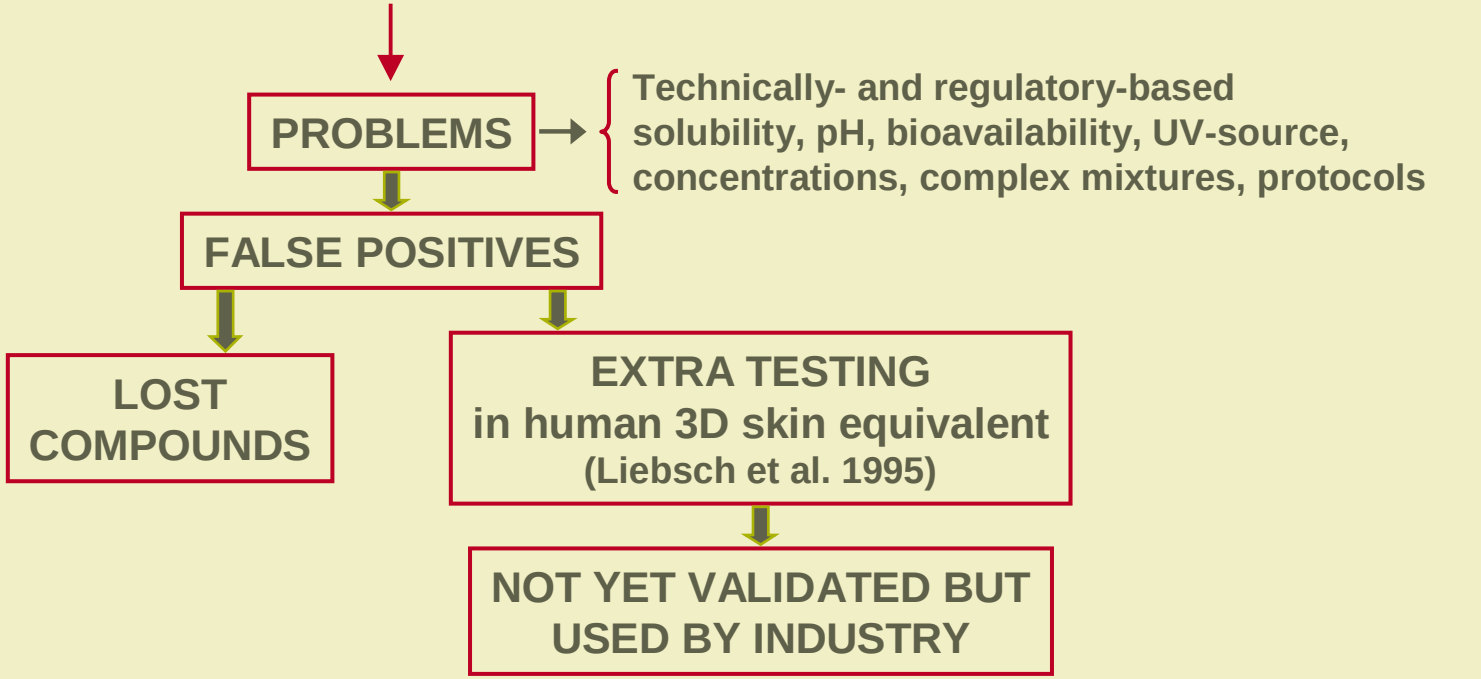
❷ 3T3 Neutral Red Uptake Phototoxicity test



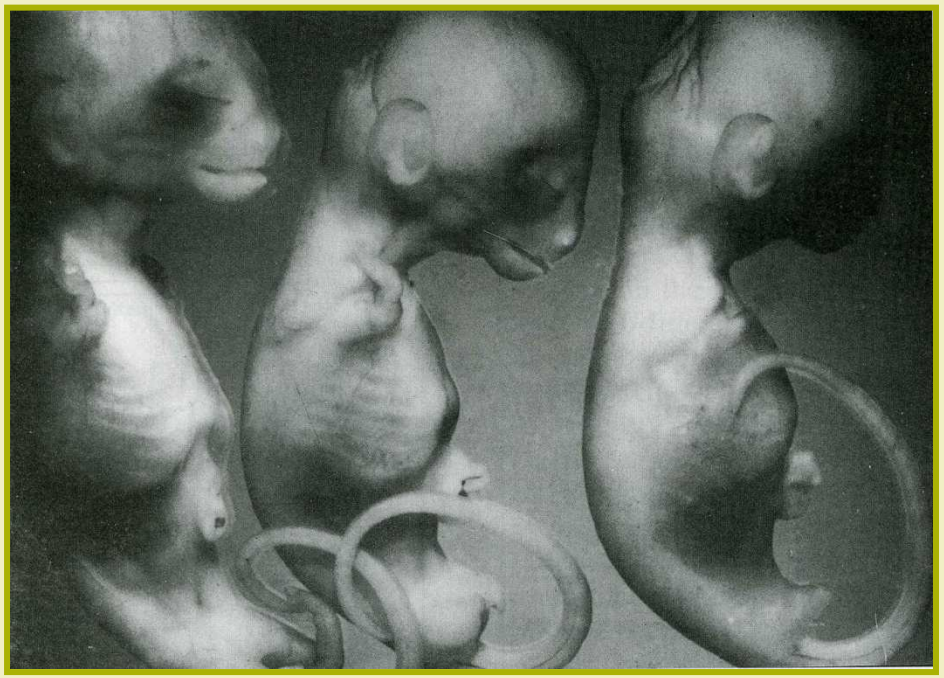
❷ 3T3 Neutral Red Uptake Phototoxicity test

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

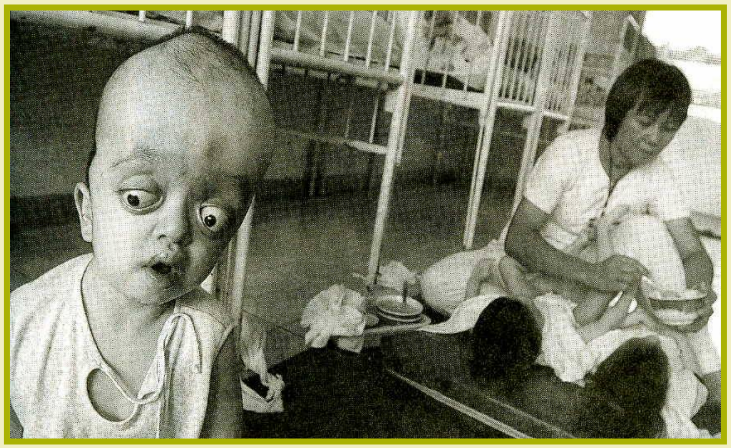
EXPERIENCE GAINED:
☺ for chemicals
☺ for UV filters (cosmetics)
☹ for pharmaceuticals (De Smet A., J&J, *epaa*, Brussels, 5/11/2007)



❸ Embryonal Stem Cell Test



Marquardt et al., Toxicology (1999)



- **Discussions about predictivity**
- **Proposal to be used as screening tool**

❸ Embryonal Stem Cell Test

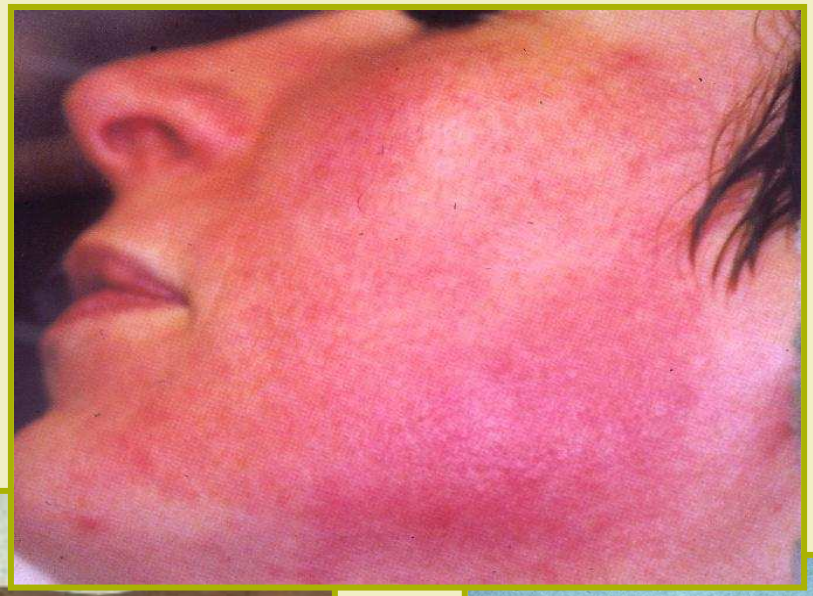
ACTUAL STATUS: ESAC approval (2001)
Regulatory refusal (???)

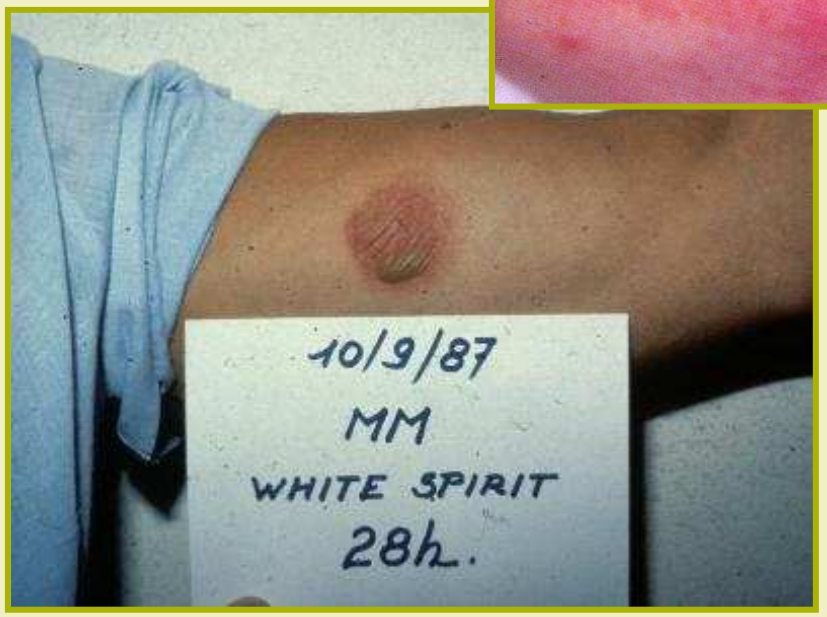
EXPERIENCE GAINED:
- ☺ for chemicals (Spielmann et al. ZEBET)
- ☹ for cosmetics: not accepted by SCCP
- ? for pharmaceuticals : ☺ or ☹ ??
  discussions about predictivity

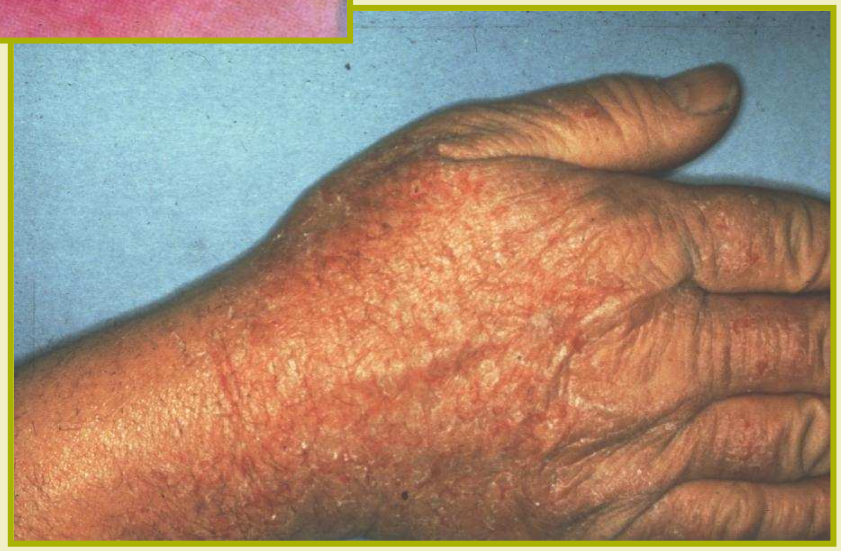
"Review on the implementation of the EST" Workshop 5/5/2008, Frankfurt

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

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







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

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 Non-irritating: 75 Slightly/mildly irritating: 23 Irritating: 11 Severely irritating: 1 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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WHICH ALTERNATIVES ARE LACKING (☹) ?

- ☹ Acute dermal toxicity
- ☹ Acute inhalation toxicity
- ☹ Eye irritation
- ☹ 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)**

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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’

CONCLUSIONS

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 DIFFERENT TYPES OF COMPOUNDS

- FOCUS ON PRIORITISATION OF THE REAL NEEDS