Skin sensitization assessment without conducting animal tests

T. Ashikaga, N. Alépée, M. Cluzel-Tailhardat, N. Gellatly, J. Hibatallah, S. Hoffmann, P. Kern, M. Klaric, J. Kühnl, S. Martinozzi-Teissier, K. Mewes, M. Millet, M. Miyazawa, D. Petersohn, M. Templier, E. van Vliet

Objective:

Regulatory accepted, animal free test strategies enabling cosmetics industry to conduct skin sensitization safety assessments

Agenda: the 4-Phase Program

Phase I:
Method Identification
and Prioritisation

Phase II:
Data Collection & Generation
Test Strategies A&D *

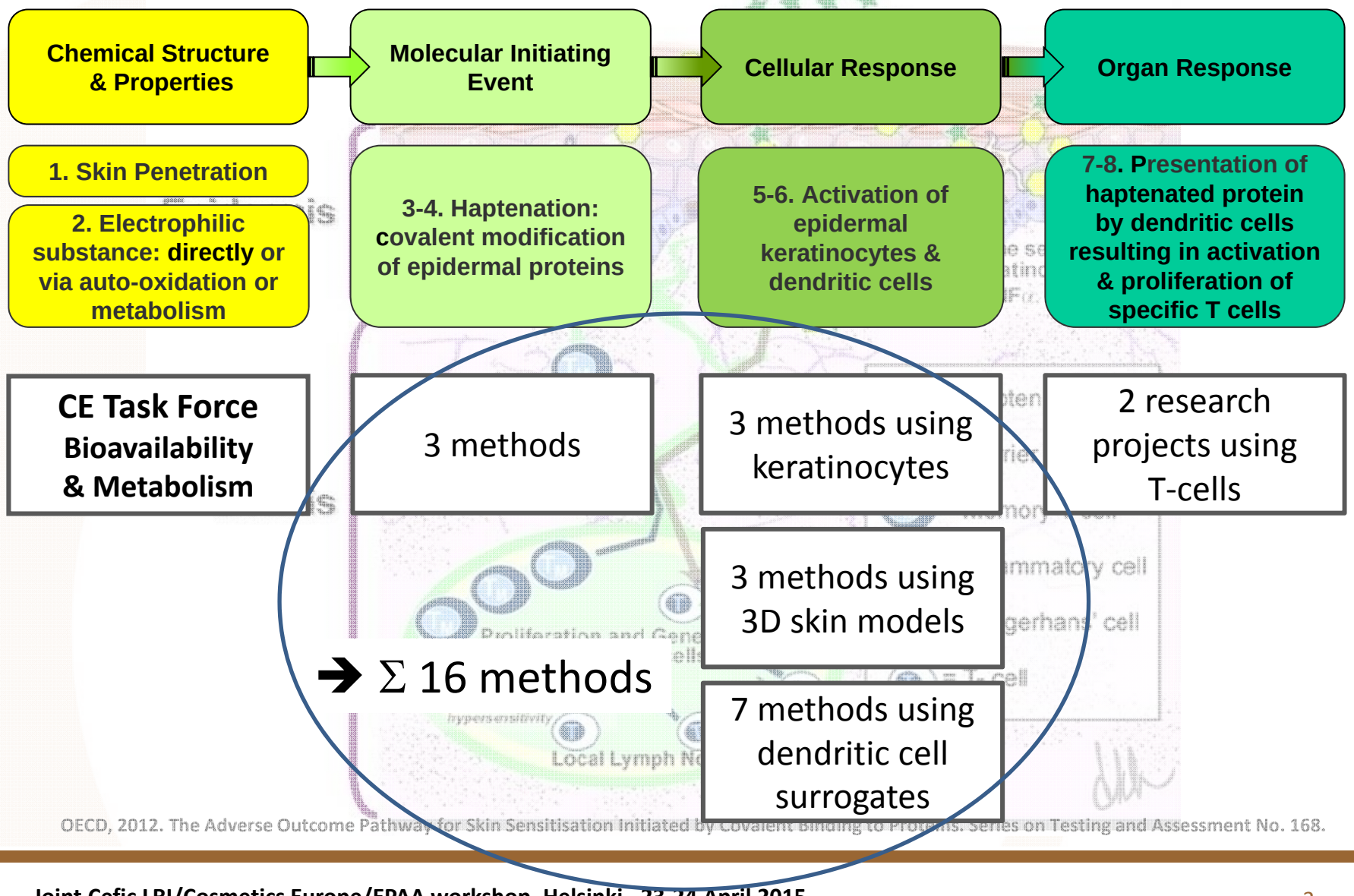
Phase III:
Assess Applicability Domain
Test Strategies Optimisation

Phase IV:
Case Studies /
Risk Assessment

*Assessment & Development

Ongoing Research Funding (e.g. T-Cells)

Phase I: Method Identification



OECD, 2012. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Series on Testing and Assessment No. 168.

Phase I: Method Prioritisation

Ref: Reisinger and Hoffmann et al. / Toxicology in Vitro 29 (2015) 259–270

Evaluation of 16 methods based on AOP

- common dataset of 10 coded substances
- Test description
- Transferability
- Reproducibility
- Predictivity
- Legal aspects
- Accessibility
- …

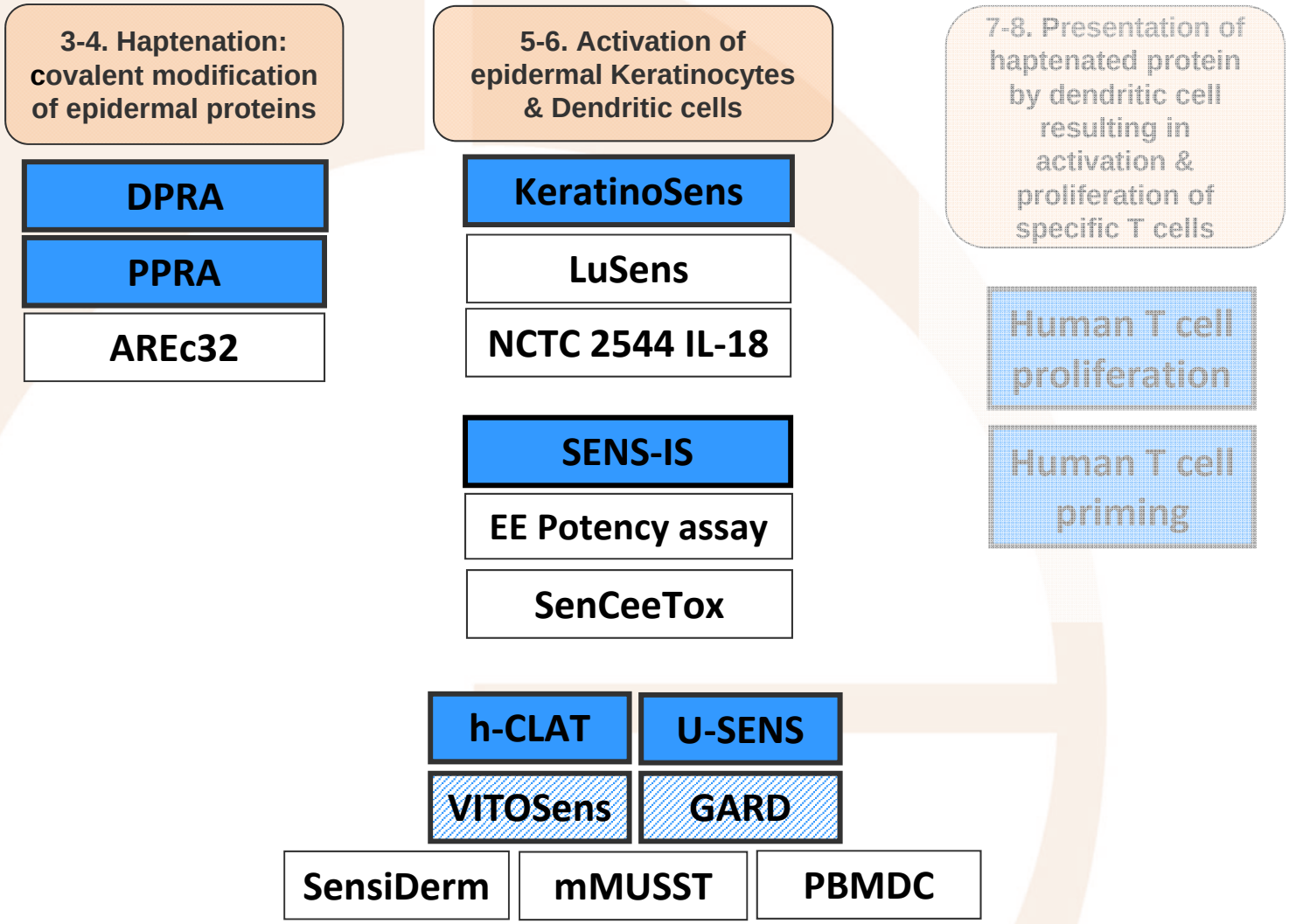
Phase I substances

	Hazard	Potency
4-Nitrobenzylbromide	S	Extreme
Methyldibromoglutaronitrile	S	Strong
Lauryl gallate	S	Strong
2-Mercaptobenzothiazole	S	Moderate
Cinnamal	S	Moderate
Tetramethyl thiuram disulphide	S	Moderate
Phenyl benzoate	S	Weak
Salicylic acid*	NS	Neg
Lactic acid	NS	Neg
Sodium lauryl sulphate**	NS	Neg / IRR

*Poorly water-soluble ** False positive in LLNA (not in human)

➔ **Outcome: 8 methods prioritized**

Phase I: Prioritized 8 Test Methods



The 4-Phase Program

Cosmetics Europe
the personal care association

Phase I:
Method Identification
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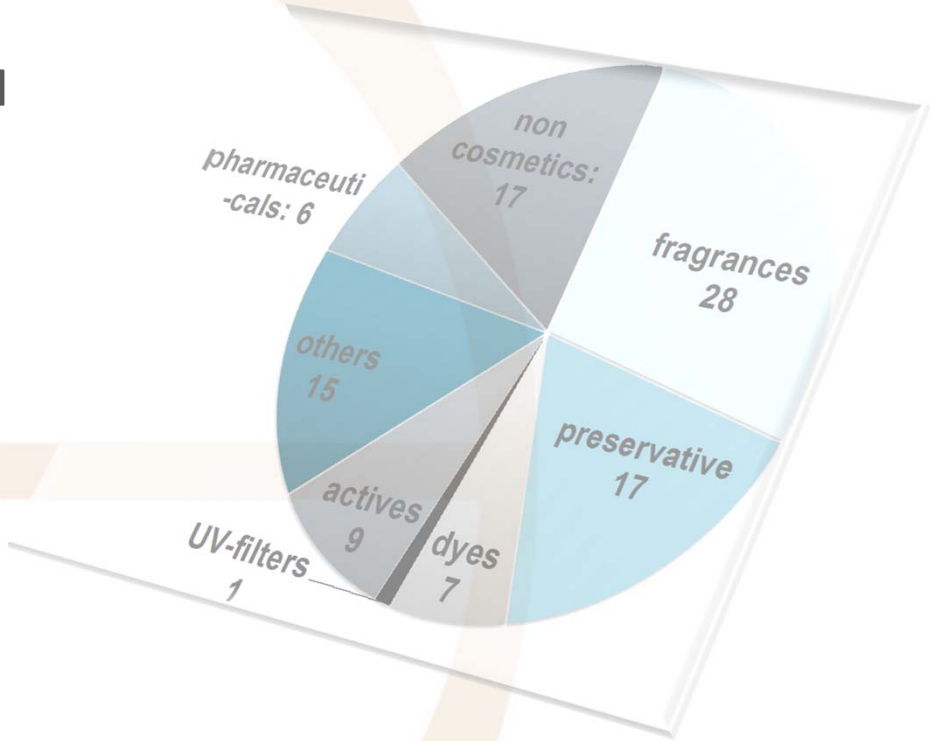
Phase II: Data Collection

Compilation of readily available information

- **Extraction of data for >100 substances (from Basketter et al. 2014)**
- **Concordant human + LLNA data**
- **6 human potency classes covered**
- **Broad chemical spectrum represented**

- **Collection of in vitro data for 8 prioritized methods**
  ➔ Set up of data matrix

- **Gap analysis of data matrix**
  ➔ Generation of new data

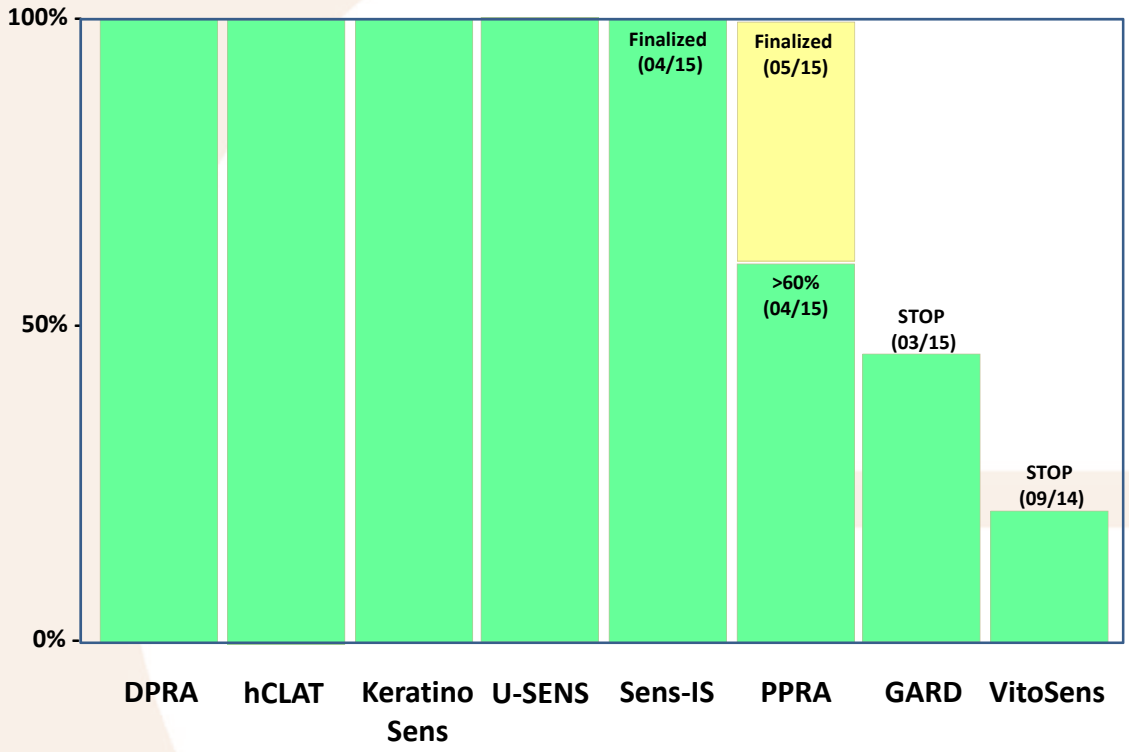


Phase II: Data Generation

to fill gaps in CE data matrix

Objective:

Generate a completed matrix of data for >100 chemicals



Next steps:

- Evaluate existing ITS's (Integrated Testing Strategies)
- Evtentually develop new ITS's

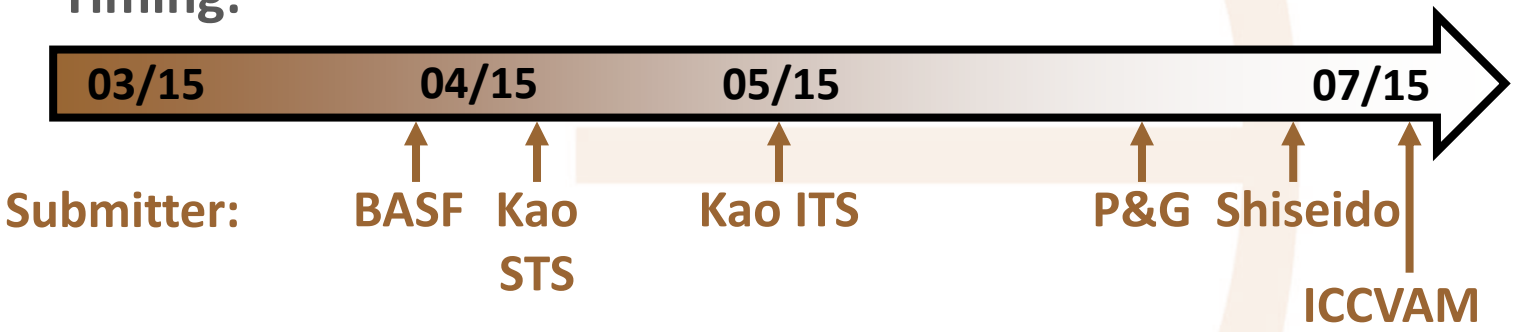
Integrated Test Strategies Evaluation

Feed CE-data into published strategies

Close cooperation with ILS / NICEATM / Idea consult:

- Transfer all data into relational Ambit data base
- Quality check and data curation
- Assessment of existing ITS‘s using naive data from CE

- Timing:



Overview: Testing Strategies

Note	Author	Title	Purpose
Assessable using CE data as input	BASF	'2 out of 3' approach	hazard ID
	ICCVAM	Integrated Approach to Testing and Assessment (IATA)	hazard ID
	Kao ITS	Score-based battery system	hazard ID
	Kao STS	Tiered system Sequential Testing Strategy	hazard ID
	P&G	Bayesian Network Integrated Testing Strategy (ITS)	potency*
	Shiseido	Artificial Neural Network for predicting LLNA EC3	potency*
Not assessable as input data missing at CE	DuPont	Implementation of an IATA into a pipeline tool (IATA-SS)	hazard ID
	Givaudan	Data from KeratinoSens and Kinetic Peptide Binding: Global Versus Domain-Based Assessment	potency*
	L'Oréal	L'Oreal's decision strategy (DS) using a "staking" meta-model	hazard ID
	RIVM	RIVM Sequential Testing Strategy	hazard ID
	Unilever	IATA for Skin Sensitisation Risk Assessment	risk assessment

*= more than three potency classes

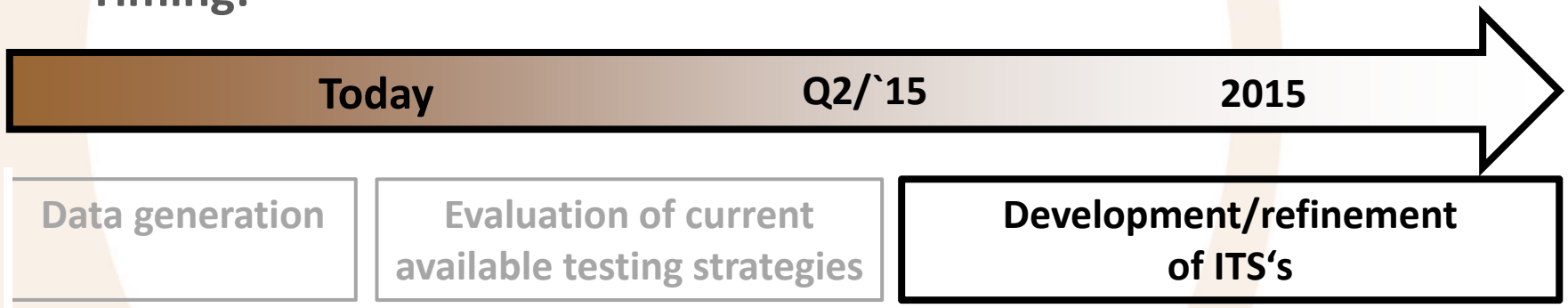
New Integrated Testing Strategies

Utilize CE-data to develop new ITS

Close cooperation with ILS / NICEATM / Idea consult:

- **If necessary: develop (and refine) independent testing strategies**
- **Build on learnings from previous ITS assessments**
- **Strategies shall be adaptable and flexible**
  (e.g. take requirements for applicability domains or new developments into account)

Timing:



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Phase III: Applicability Domain and Testing Strategies Optimisation

Testing of especially cosmetic ingredients:

- **Chemicals of utmost importance for cosmetic industry** (e.g. hair dyes, UV-filter, preservatives, natural extracts)

- **Especially challenging physico-chemical properties**

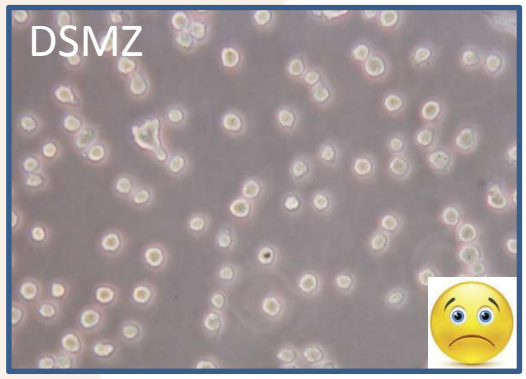
Status:

- **List of chemicals finalized**
- **Testing contracted and data generation started**

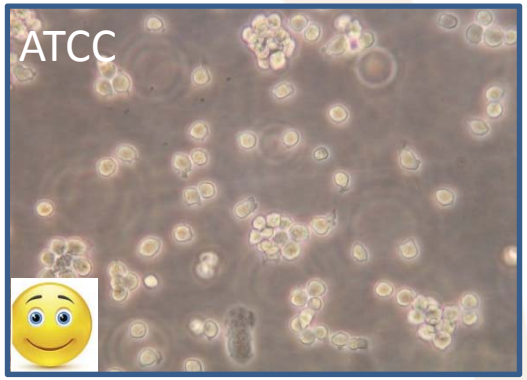
Phase III:
Assess Applicability Domain

Some issues identified:

- **h-CLAT: Integrity of THP-1 cells from different sources**



- Dead cells / thawing (+++)
- Recovery phase (2-3 weeks)
- Vitality, untreated (≤ 90%)



- Dead cells / thawing (+)
- Recovery phase (1 week)
- Vitality, untreated (≥ 90%)

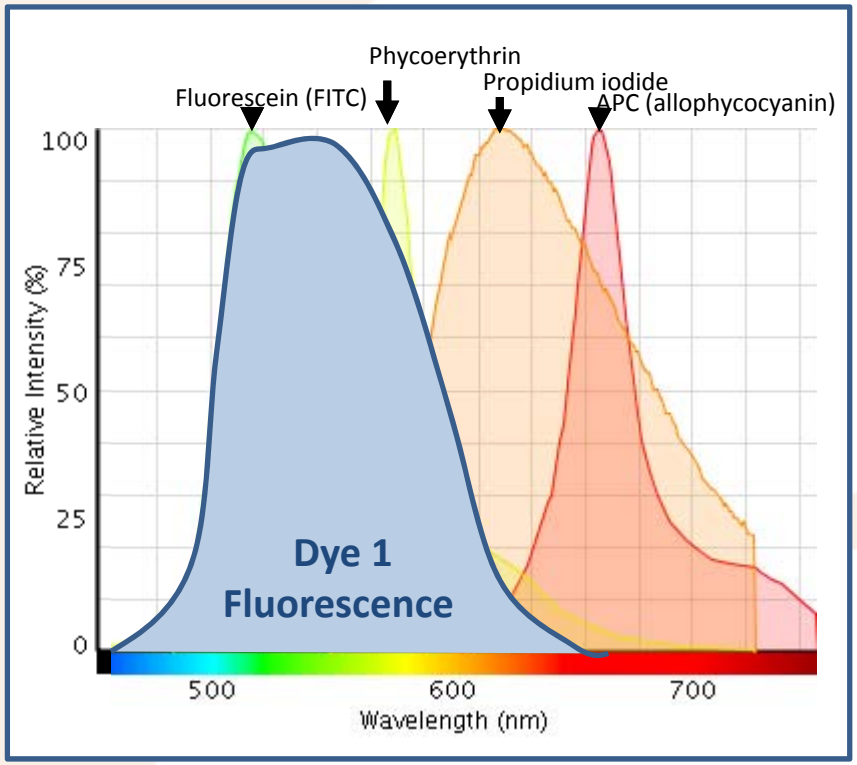
Reco

➔ Use the cells which meet the acceptance criteria in accordance with SOP

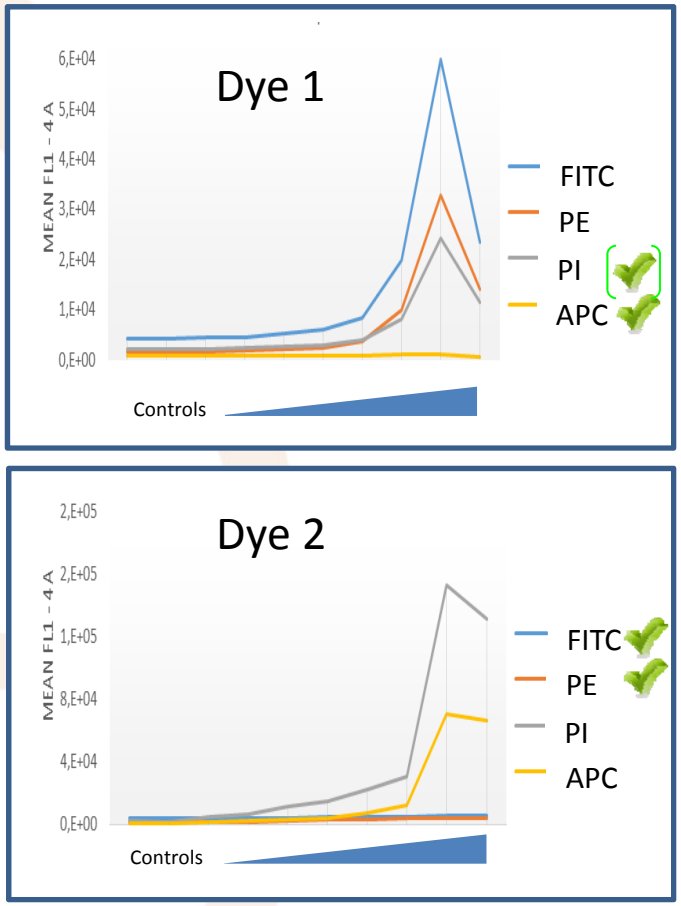
Phase III:
Assess Applicability Domain

Some issues identified:

- Fluorescence interferences



Fluorescence check



Initial learnings

- Sufficient quality check for cells

- Fluorescence interferences:
  - ➢ Typical fluorescent substances (e.g. p-phenylenediamine) were predicted correctly using h-CLAT (Okamoto et al., AATEX, 2010)

  But

  - ➢ Strong fluorescent substances need special care, i.e. fluorescence checks, use of non interfering fluorescent labels

  Reco

  ➔ Confirm that available fluorescent labels can provide similar results as FITC label, by testing proficiency substances!

- Quenching effects from e.g. dyes when using luminescence assays (tbc) ?

The 4-Phase Program

Phase I:
Method Identification and Prioritisation

Phase II:
Data Collection & Generation
Test Strategies (A&D)

Phase III:
Assess applicability domain
Test Strategies Optimisation

Phase IV:
Case Studies / Risk Assessment

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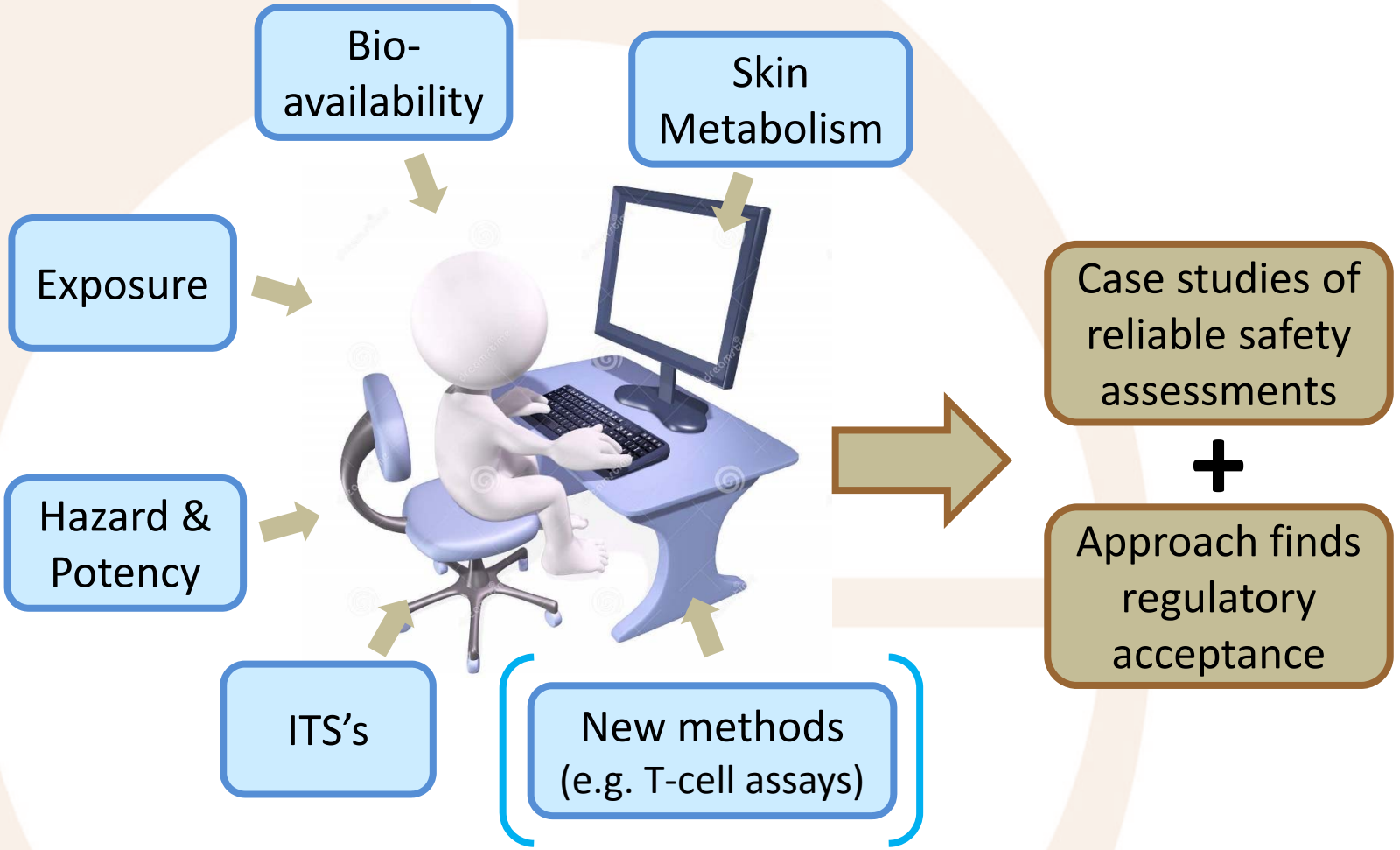
Case Studies / Risk Assessment



Thank you for your attention!