

Example for a corrosive amine classified false-negative in an *in vitro* skin irritation test

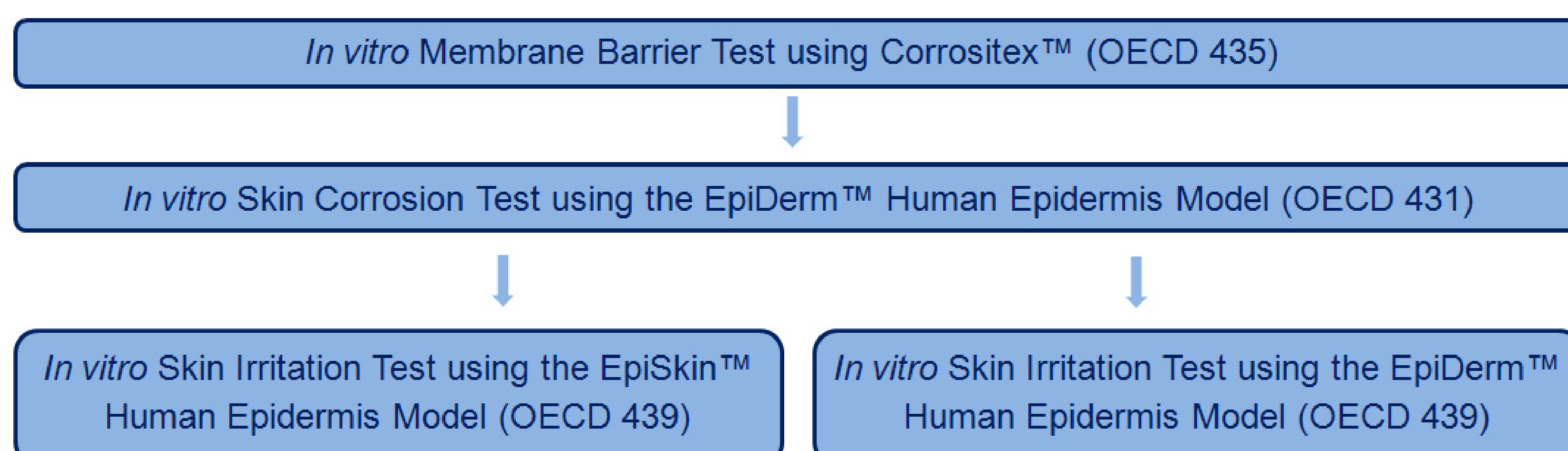
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Introduction

Considering animal welfare concerns alternative *in vitro* test methods for skin corrosion/irritation testing have been validated and accepted as full replacement tests by applying a tiered testing strategy (see Annex to OECD 404, 2002). We examined one amine derivative to determine best suitable test methods for classification of several substances of the same chemical class using the following methods in consecutive steps:



Methods

In vitro Membrane Barrier Test (OECD 435):

- CORROSITEX™ Assay Kit (InVitro Int., USA):
 - synthetic macromolecular BIOBARRIER matrix
 - chemical detection system (CDS)
- Initial categorization test:
 - category 1: high acid/alkaline reserve
 - category 2: low acid/alkaline reserve
- Corrosive materials are identified by their ability to pass through the bio-barrier membrane after topical application and to elicit a colour/structure change in the underlying CDS
- Penetration time is used for subcategorization according to UN GHS:

Category	Time [min]	Time [min]	Time [min]	Time [min]
1	0 - 3	> 3 - 60	> 60 - 240	> 240
2	0 - 3	> 3 - 30	> 30 - 60	> 60
UN GHS Subcategory	CORROSIVE 1A	CORROSIVE 1B	CORROSIVE 1C	Non Corrosive

In vitro Skin Corrosion Test (OECD 431):

- EpiDerm™ RHE (MatTek Corp., USA)
 - reconstructed epidermis with a functional stratum corneum
- Topical application, treatment for 3 min. and 60 min.
- Determination of cell viability by the MTT reduction assay
- Prediction Model:

Mean tissue viability (% negative control)	Prediction
3 min: < 50%	CORROSIVE
3 min: ≥ 50% and 60 min: < 15%	CORROSIVE
3 min: ≥ 50% and 60 min: ≥ 15%	NON-CORROSIVE

In vitro Skin Irritation Tests (OECD 439):

- **Model 1:** EpiDerm™ RHE (MatTek Corp., USA)
- **Model 2:** EpiSkin™ RHE (SkinEthic Laboratories, France)
 - reconstructed epidermis with a functional stratum corneum
- Topical application, treatment for 60 min. (EpiDerm™) or 15 min. (EpiSkin™)
- Determination of cell viability after 42 h post-incubation by the MTT reduction assay
- Prediction Model:

Mean tissue viability (% negative control)	Prediction
≤ 50 %	IRRITANT
> 50 %	NON-IRRITANT

Direct MTT reduction potential was tested prior to treatment
Treatment of freeze-killed tissues for quantitative correction of results

Results

Category 1 Mean time: 110 min.	CORROSIVE Subcategory 1C
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3 min.: 95% 60 min.: 13%	CORROSIVE
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Model 1: EpiDerm™ 5.9%	IRRITANT
Model 2: EpiSkin™: 99.8%	NON-IRRITANT

Direct MTT reduction potential < 5%

Conclusions

- The corrosive amine derivative was classified false-negative in the *in vitro* skin irritation test using the EpiSkin™ RHE model, representing an example for the chemical class of amines showing a tendency for misclassification in the *in vitro* skin irritation test as stated by the ICCVAM 2009

- For amine derivatives, a Top-Down strategy or a Bottom-Up strategy using the EpiDerm™ RHE model seem to be suitable testing strategies for classification. A Bottom-Up strategy using the EpiSkin™ RHE model for skin irritation testing bears the risk of misclassification

