Development of an Animal Product-Free Acute Toxicity Screen

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INTRODUCTION

Acute toxicity is a key human health endpoint, requiring assessment by many global chemical safety regulators. Animal-based methods are used for acute toxicity with no validated alternative available or in development. XCellR8 has developed an acute toxicity screen using animal-product-free conditions. Comprised of a modified neutral red uptake (NRU) method and prediction model, we have screened 20 cosmetic ingredients to produce IC_{50} values (concentration where cell viability is 50%). Through analysis of IC_{50} values with the prediction model, 13/18 blind-tested method compatible chemicals were placed into the same GHS category as that derived from *in vivo* acute toxicity studies. This *in vitro* acute toxicity test is proposed as the initial step of an integrated approach to testing and assessment, incorporating multiple organ-specific endpoints. As a next step, XCellR8 aims to model metabolism in the current test and promote its utility for other chemical sectors.

METHODS

CELL CULTURE

Human dermal fibroblasts (HDFn) in Fibrolife™ Xeno-free medium – maintained at 37 °C/5% CO2

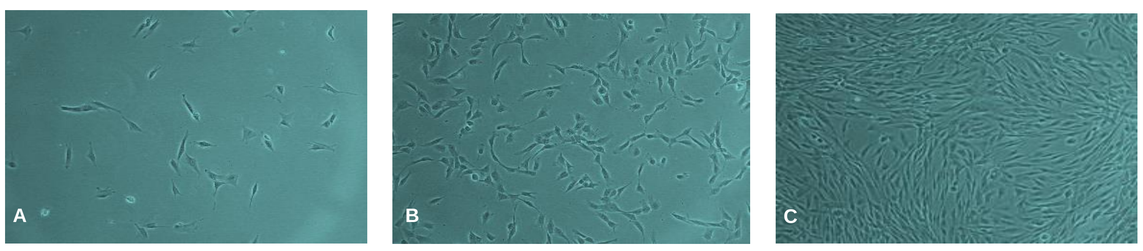

Figure 1: Images of HDFn-XF cells at passage 1, day 1 (A), day 2 (B), and day 4 (C) x10 magnification

ASSAY

- **Solubility** tests followed OECD Test Guidance document 129 for **20** ingredients of varying known toxicity levels from five functional cosmetic groups
- **Cells** seeded into 96-well plates and treated with 8 different concentrations of each test sample. A range-finding test was followed by a main experiment to determine **IC_{50}** (concentration where cell viability is 50%).
- **Neutral Red uptake** by the cells was determined by reading the absorbance at 540nm with a spectrophotometer. The data was used to determine the **IC_{50}** , allowing each chemical to be placed into a GHS category via a prediction model.

RESULTS

Table 1: IC_{50} values of 20 test items and their in vivo/vitro toxicity categories. Items highlighted green showed concordance between classifications.
** Methylparaben and propylparaben had very close IC_{50} and IC_{20} values, so were classified as false positives. The closer these values are together, the more likely the potential for toxicity*

Test Item	GHS oral tox. classification	*In vitro* acute tox. classification	IC_{50} Value (µg/ml)
Ammonium Lauryl Sulfate	4	4	129
Sodium Laureth Sulfate	4	4	150
Cocamidopropyl Betaine	4	4	67.8
Stearic Acid	5	4 or above (solubility limitation)	>100
Benzyl Acetate	5	5	>2000
Lemon Oil	5	4	306
Limonene	5	N/A (Too volatile)	<8000
Pulegone	4	5	>2000
Methylparaben	5	4*	352
Propylparaben	N/A	4*	155
Sodium Benzoate	5	5	22187
Phenoxyethanol	4	5	2205
Tudor Ebony (Black 11)	5	4 or higher	>100
Tudor Paeony (Red 7)	5	4 or higher	>200
Titanium Dioxide-Nano	N/A	5 or higher	>2000
Octyl Methoxycinnamate	5	5	>2000
Dimethicone	5	5	>200000
Glycerin	N/A	5	147172
Lanolin	N/A	N/A (solubility limitation)	>20
Triethanolamine	5	5	3364

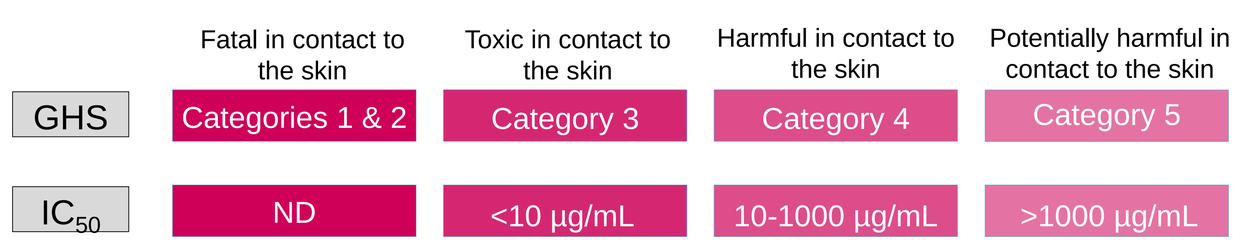

Figure 2: Prediction model for in vitro acute toxicity following determination of IC_{50} values of 20 test items

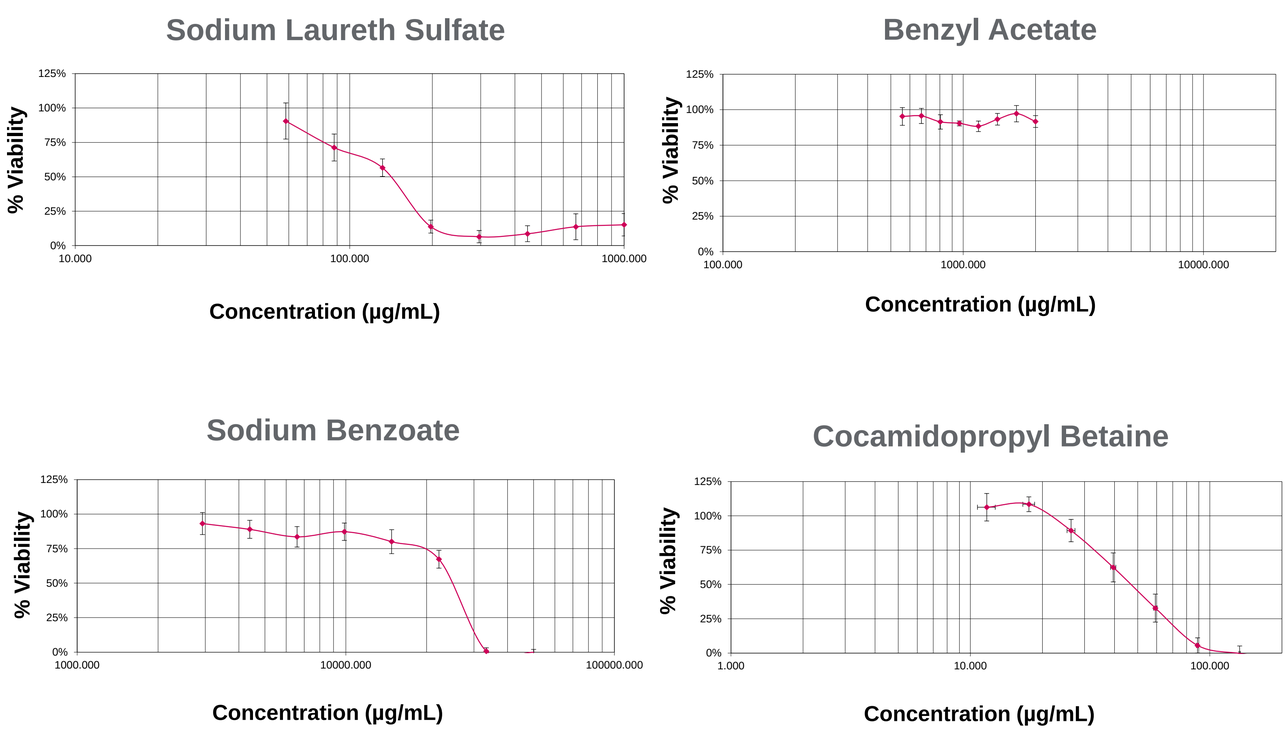

Figure 3: Evaluation of cellular viability by neutral red uptake for four test items

DISCUSSION

The results show 13 chemicals for which *in vitro* IC_{50} values were determined and were placed into the same GHS classification as that based on LD_{50} data obtained by oral acute toxicity. Removing the chemicals for which no IC_{50} could be determined due to solubility limitations, this resulted in the test correctly classifying 13/18 chemicals (72%). Cytotoxicity is a key event in modes of action associated with acute health effects, covering mechanisms of toxicity common to most cell types that can lead to organ failure, including disruption of membrane function – the endpoint measured in the neutral red uptake test. We envisage that a robust human cytotoxicity assay would constitute an early-stage screen for highly toxic chemicals. Chemicals that are not classified as highly toxic in the cytotoxicity pre-screen would then be tested further using organ-specific endpoints. XCellR8 will seek to incorporate the study of metabolism into the assay, in addition to refining the prediction model. It is hoped this acute toxicity screen will form the first stage of an integrated *in vitro* testing strategy for acute toxicity.

REFERENCES

Hoffmann *et al.* (2010). Acute oral toxicity: variability, reliability, relevance and interspecies comparison of rodent LD50 data from literature surveyed for the ACuteTox project. Regul Toxicol Pharmacol. 58(3): 395-407.
Kinsner-Ovaskainen, A. *et al.* (2013). Selection of test methods to be included in a testing strategy to predict acute oral toxicity: an approach based on statistical analysis of data collected in phase 1 of the ACuteTox project. Toxicology In Vitro. 27(4): 1377-94.