Non-animal safety assessment approaches for cosmetic formulations and ingredients based on 3T3 NRU test

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Introduction:

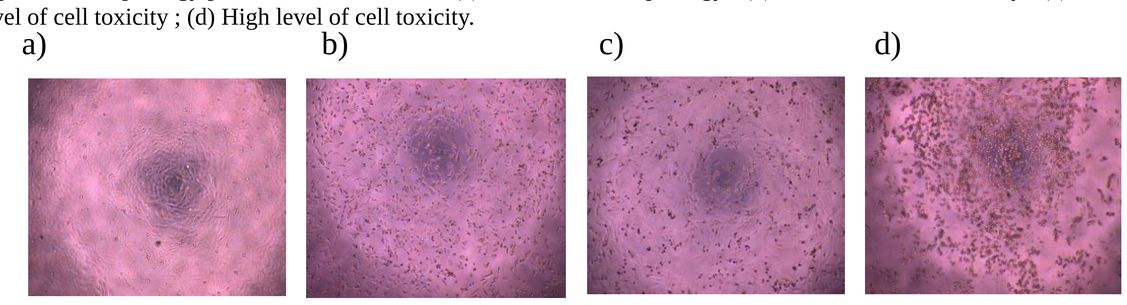
In order to ensure animal welfare and the safety of consumers, alternative techniques were employed in cosmetic toxicology. Safety assessment of cosmetics requires further introduction of alternative methods into the tier testing strategy [1]. The 3T3 neutral red uptake (NRU) is a method using cell viability to evaluate basal cytotoxicity. We have established this method for the safety assessment of raw materials and finished products. The safety assessment begins with physico-chemical evaluation of the products and based on existing toxicologic data of the similar ingredients and products, followed by in vitro testing, and finally a confirmation test on human volunteers [2]. A cytotoxicity assay, combined with human patch test, can ensure the safety of finished cosmetic products. Therefore, 3T3 NRU is effective and low-cost as the first step in safety assessment.

Materials & Methods:

Balb/c 3T3 fibroblasts (ATCC CCL-163) according to the protocol DB-ALM 46&64 [3,4] were cultured in dulbecco's modified eagle medium (DMEM, Gibco) supplemented with 10% bovine serum at 37°C, with 5% (v/v) CO_2, subcultured in 96-well plates. The cells were seeded into 96-well plates (100 µl medium with 100 µl of a 7–10 × 10^4 cells.ml^{-1} cell suspension per well), and incubated for 24 hours, in order to form a 60–70% confluent monolayer. After removal of the growth medium, test substances were added to the cells (100 µl/well), with blank controls treated with DMEM alone, and positive controls treated with sodium lauryl sulphate. Samples were dissolved in suitable solvent, diluted in culture medium with 5% bovine serum.

The cells were exposed to the tested materials for 24 h. Record any changes in morphology of the cells due to the cytotoxic effects of the test substance, scoring followed Fig 1. These records form circumstantial evidence, but can not to be used for any quantitative measure of cytotoxicity. Afterwards, NR working solution is added to the wells. After 3 hours incubation, the solution is removed, washing cells with Hank's Balanced Salt Solution (HBSS) and a desorbing fixative (ethanol/acetic/water) is added for 30-45 minutes. NR taken up by the viable cells is extracted and the absorption is measured in a microplate reader (eon, bioteck, USA) at 540 nm.

Fig 1. Cell morphology photos at different levels (a) Normal cell morphology ; (b) Low level of cell toxicity ; (c) Moderate level of cell toxicity ; (d) High level of cell toxicity.



Results & Discussion:

We tested different types of products, including 12 toners, 11 lotions, 15 serums, 12 face creams, 12 shampoos and 12 hair conditioners, results showed in safety limits was established by evaluating the cell viability data under the treatments of products at different concentrations in Fig 2.

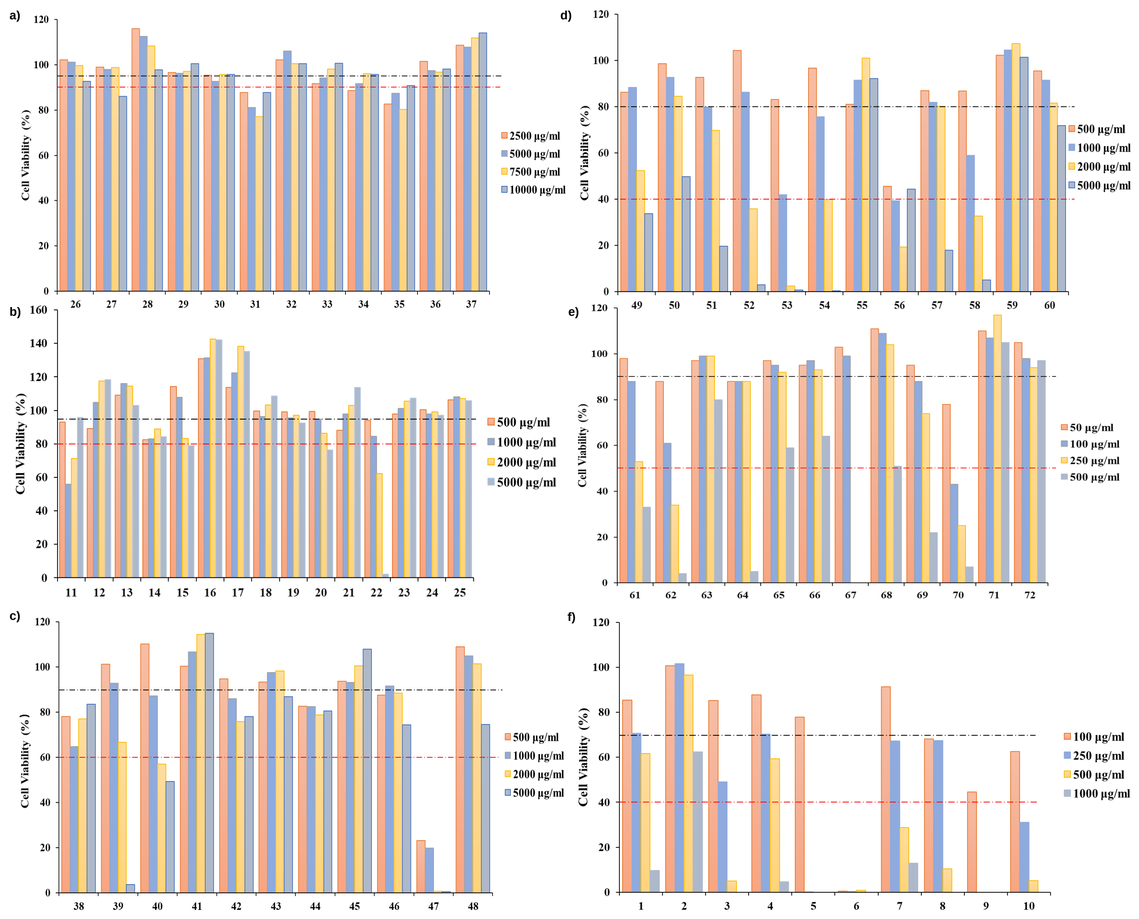


Fig 2. Cell viability at four concentrations of different types of products: (a) toners, (b) serums, (c) lotions, (d) face creams, (e) shampoos, (f) hair conditioners.

At the same time, the mildness standard is established to support the mildness claims of products (Table 2).

Table 2. Safety and mildness cell viability limits of different types of products

Products Category	Concentration (µg.ml^{-1})	Cell Viability (%)	
		Safe	Mildness
Toners	5000	⩾90	⩾95
Serums	2000	⩾80	⩾95
Lotions	1000	⩾60	⩾90
Face Creams	1000	⩾40	⩾80
Shampoos	100	⩾50	⩾90
Hair Conditioners	100	⩾40	⩾70

Conclusions:

3T3 NRU is a low-cost, efficient and high-throughput testing method that can be used as the first step in product safety assessment strategy. The cytotoxicity data of raw materials can provide evidence for the level added in formulation. After testing of hundreds of cosmetics, we have established a standard of safty limits for our laboratory the range of cytotoxicity specific for individual categories of cosmetics. As the database expands, it can provide evidence for mildness claim of finished products.

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