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IVIV

(In Vitro-In Vivo extrapolation) approach to estimate doses for acute oral systemic toxicity tests

A cell biologist with 8 years of experience *in in-vitro* assay development and GLP validations. She is actively involved in developing various assays in the areas of sensitisation, endocrine disruption, generation of 3D models and is a key member in technology transfer.

Introduction

The 3R principles (Reduce, Refine, Replace) strongly emphasizes on replacing the animal models as far as possible with non-animal alternatives. In case of the *in vivo* oral toxicity screening of compounds, traditional approaches such as Acute Toxicity Class (ATC), Up and Down Procedure (UDP), Fixed Down Procedure (FDP) are employed. Various *in vitro* alternative methods are available that can if not fully replace but, reduce the number of animals. One such approach is a part of the OECD guidance document 129 entitled "Guidance Document on using Cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests", which describes a cytotoxicity test using BALB/3T3 murine fibroblast cells. Cellular toxicity is determined by the ability to uptake the neutral red dye. These effects seen in *in vitro* models can be directly extrapolated to the *in vivo* outcome.

The objective of this method is to eliminate the need of testing the lethal doses on animals, in turn reducing the number of animals by 25-40%. IC_{50} value of a test compound generated from such *in vitro* cytotoxicity methods can be used to estimate LD_{50} value. This value can be further used to eliminate the number of toxic doses to be tested on animals.

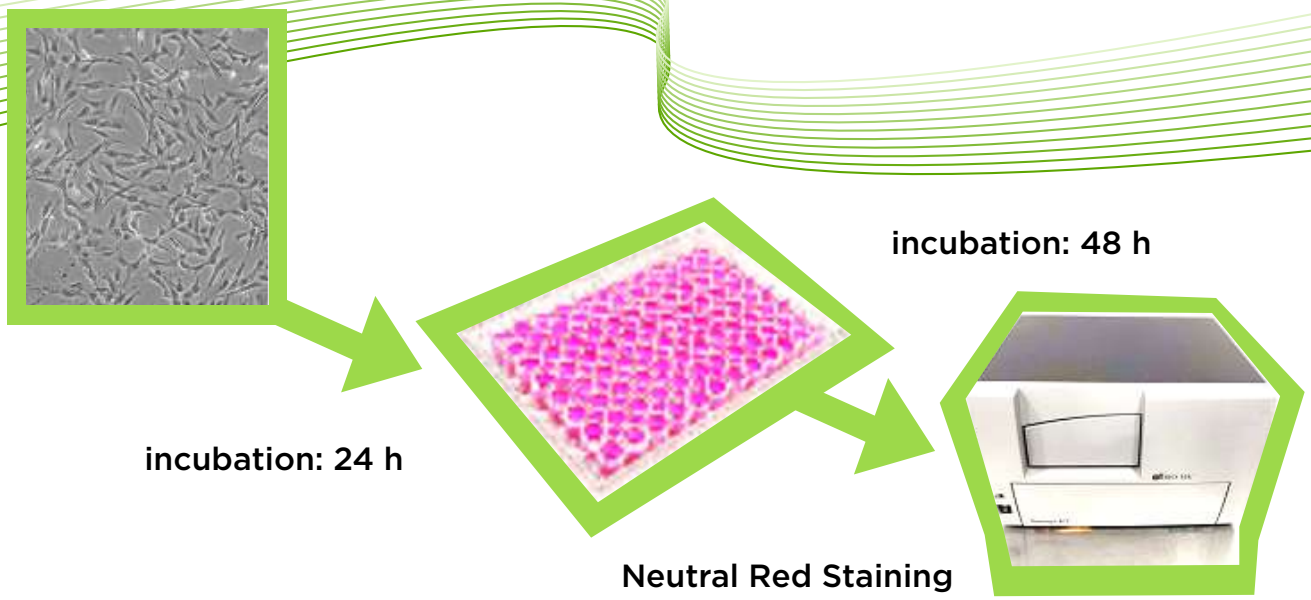
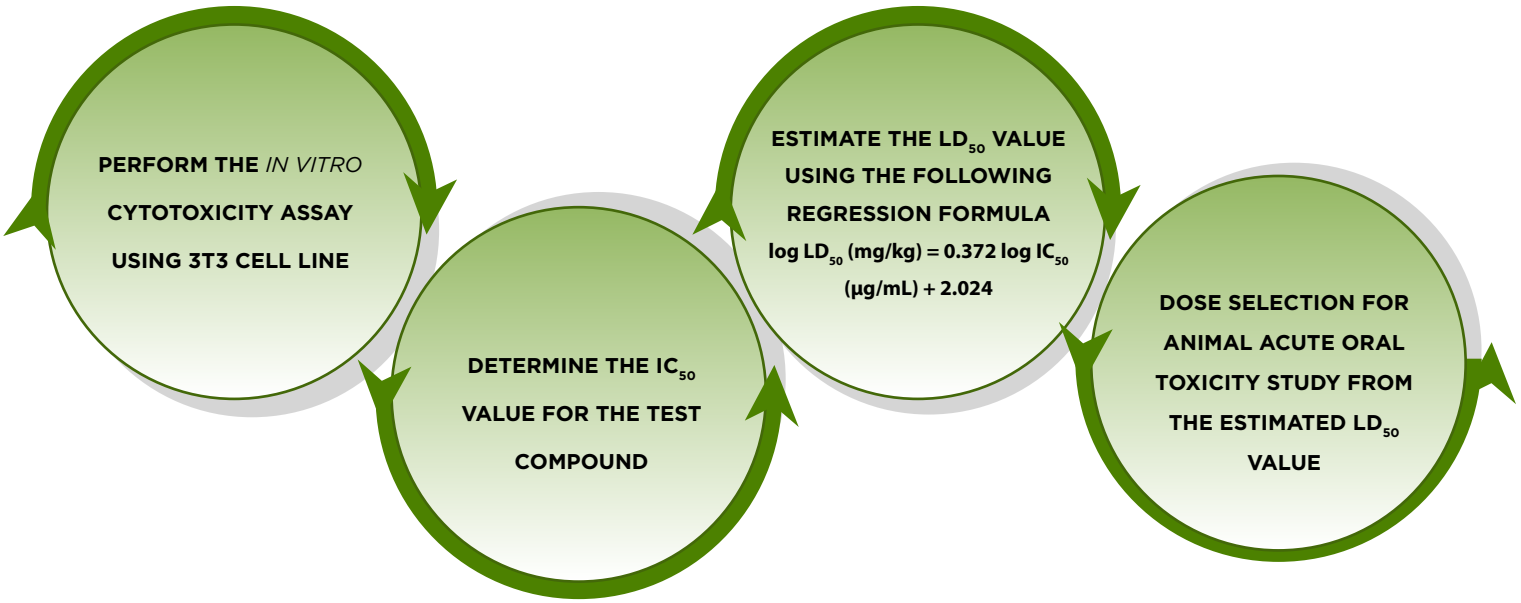


Figure: Schematic representation of Neutral Red Uptake (NRU) assay setup

We at Jai Research Foundation are committed to eliminating the unnecessary use of animals in compliance with 3Rs. Thus, we are happy to offer this *in vitro* model based on OECD GD 129 to reduce animal usage in acute oral systemic toxicity.

Typical workflow



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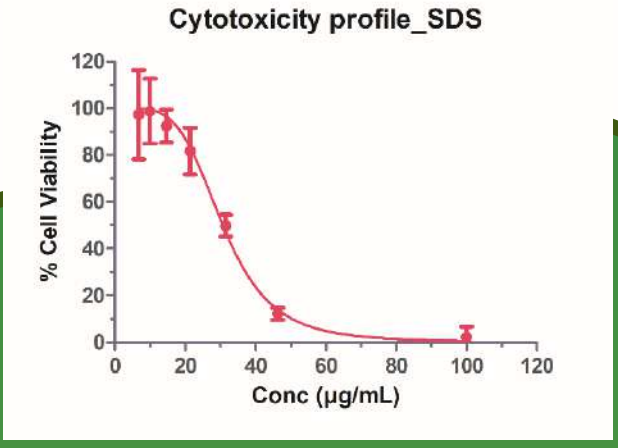

Figure: Dose response curve of cells upon treatment with test compound compound (SDS) followed by staining with neutral red.

We have tested the cytotoxic effect of Sodium Dodecyl Sulfate (SDS), positive control at different concentrations and have found the compound to be highly cytotoxic with an IC_{50} (inhibitory concentration reducing the cellular viability by 50%) of 33.6 µg/mL. Using IC_{50} value, the LD_{50} value of the test compound was estimated and was found to be 390.8 mg/kg, which is then used to estimate doses for acute oral systemic toxicity tests.

Test Chemical	IC_{50} value (µg/mL)	LD_{50} value (mg/kg)	Final Prediction
SDS	33.6 ± 2.4	390.8 ± 10.2	Cytotoxic

The table represents the mean IC_{50} and LD_{50} values of the test compound SDS. The test compound was exposed to BALB/3T3 cells at 8 different test concentrations (6.7 – 100 µg/mL) and incubated for 48 h, before neutral red staining (for determining the cytotoxicity).

Benefits/Application of this approach

The LD_{50} value estimated from the *in vitro* cytotoxicity assay can be of help in minimising the number of doses to be tested in acute oral systemic toxicity tests. For example, in the case of SDS,

- IC_{50} value = 33.6 µg/mL
- log LD_{50} (mg/kg) = 0.372 log IC_{50} (µg/mL) + 2.024
- log LD_{50} (mg/kg) = (0.372 * 1.526) + 2.024
- log LD_{50} (mg/kg) = 2.592; Therefore, LD_{50} = 390.8 mg/kg

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Dose Selection In UDP Test Method:

Recommended doses in animal oral testing

Limit test of 2000 mg/kg = 1.75, 5.5, 17.5, 55, 175, 550, and 2000 mg/kg
Number of animals used = 3*7 doses =21 animals

Limit test of 5000 mg/kg = 1.75, 5.5, 17.5, 55, 175, 550, 1750, and 5000 mg/kg
Number of animals used = 3* 8 doses =24 animals

Estimated LD_{50} value for SDS = 390.8 mg/kg

As per estimated LD_{50} following doses should be used in rats for acute oral testing:

For the Limit test of 2000 or 5000 mg/kg = 1.75, 5.5, 17.5, 55, 175 mg/kg (one default dose below the estimated LD_{50} value)

Number of animals used = 3*5 doses =15 animals

Dose selection in ATC and FDP test methods

Default doses used in animal (rats) oral testing

Recommended doses in animal oral testing

Limit test of 2000 mg/kg = 5, 50, 300, or 2000 mg/kg
Number of animals used = 3*4 doses =12 animals

Limit test of 5000 mg/kg = 5, 50, 300, 2000, 5000 mg/kg
Number of animals used = 3*5 doses =15 animals

As per estimated LD_{50} following doses should be used in rats for acute oral testing:

For Limit test of 2000/5000 mg/kg (in case of both ATC & FDP methods) = 5, 50, 300
(one default dose below estimated LD_{50} value)

Number of animals used = 3*3 doses = 9 animals

Thus, this approach automatically eliminates screening of the higher doses of 550 and 2000 mg/kg of SDS in rats. Thus, when using the IVIVC *(in vivo-in vitro* correlation*)* approach, there is animal saving of around 25% in acute toxicity methods with toxic compounds.

Conclusion

Acute toxicity method	Limit test (mg/kg)	No. of animals used	Number of animals to be used (after application of OECD GD 129 method)	Animal reduction (%) for toxicity testing of SDS
UDP	2000	21	15	29
	5000	24		38
ATC/FDP	2000	12	9	25
	5000	15		40

Thus, this approach of utilizing the *in vitro* 3T3 NRU cytotoxicity experimental data in predicting *in vivo* phenomena (i.e., estimating doses for rodent acute oral toxicity tests) can help in reducing the number of animals tested in an *in vivo* study in a significant manner.

References

1. OECD Environment, Health and Safety Publications, Series on Testing and Assessment, Number 129, 2010: Guidance Document on using cytotoxicity tests to estimate starting doses for Acute Oral Systemic Toxicity Tests, adopted on 20 July 2010.
2. ICCVAM Test Method Evaluation Report: *In Vitro* Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests" NIH Publication No. 07-4519, Published 2006.