Leading Ecotoxicology team! With over 17 years of experience in the CRO industry, he brings a wealth of knowledge in both aquatic and terrestrial studies. Dr. Jigar has played a pivotal role in validating Ecotoxicity studies and is an active member of the Society of Toxicology.

Dr. Jigar R. Rana,
Group-Leader Ecotoxicology

Fish Cell Line Acute Toxicity: The RTgill-W1 Cell Line Assay using 3,4-Dichloroaniline

INTRODUCTION

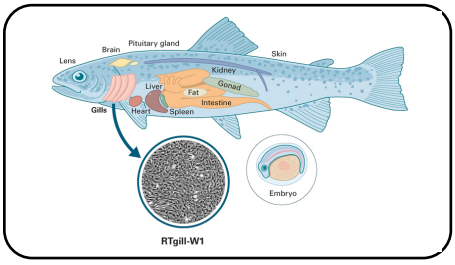
The most used animal test for environmental risk assessment is the acute fish toxicity test [1]. This test requires a considerable number of fish, 42-60, takes five working days (exposure period), produces litres of toxic waste and uses death as the integrative but crude endpoint. Millions of fish are used annually for regulatory requirements and whole effluent testing. *In vitro* models have been beneficial in predicting the toxicity of chemicals. The gills of aquatic organisms are unique organs involved in gas exchange, osmoregulation, and other critical functions essential for the survival of fish and invertebrate species [2]. The gills of aquatic organisms are the primary target and uptake sites of water contaminants. As such, gills are exquisite organs for the study of aquatic toxicant effects [3]. However, gills *in vivo* are difficult to evaluate or manipulate. Thus, gill cells *in vitro* could represent ideal systems for studying aquatic contaminants. RTgill-W1 is a permanent cell line from rainbow trout *(Oncorhynchus mykiss)* gill.

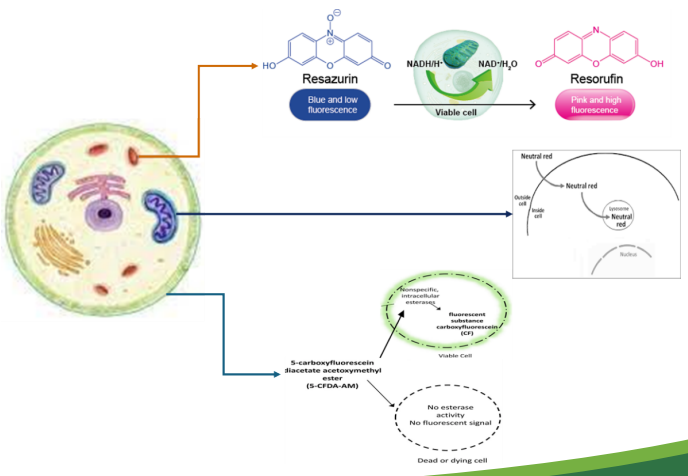
As an alternative method for fish acute toxicity test, we used a gill cell line from rainbow trout *(Oncorhynchus mykiss)*, RTgill-W1 [4, 5]. The RTgill-W1 cell line assay is an *in vitro* alternative to traditional fish acute toxicity testing [5]. In this cell line assay, the need for fish is entirely omitted; therefore, it offers an impactful alternative in terms of the 3Rs. It has the same fields of application as the fish acute toxicity test. These fields include

(1) the determination of an EC value for fish cell viability as a potential surrogate for acute fish toxicity;
(2) range-finding and pre-screening before conducting a full fish acute or other fish-based toxicity test;
(3) generation of toxicity information to be used for hazard assessment [5].
In this study, confluent monolayers of the RTgill-W1 cell line of three different passages (P18, P21, and P24) were exposed at a range of 3.13 to 100 mg DCA/L for 24 h in 24-well tissue culture plates as recommended in OECD 249.

RTgill-W1 Cells in confluent monolayers in 24-well cell culture plates are exposed to the test item for 24 h in the dark → Cell viability is assessed with a set of fluorescent cell viability indicator dyes. → EC_{50}, NOEC and LOEC (where possible)

MATERIALS AND METHODS
Test Item: 3,4-dichloroaniline (97.6%), Sigma-Aldrich, Switzerland
Test System: RTgill-W1 cell line (CRL-2523, ATCC, USA)





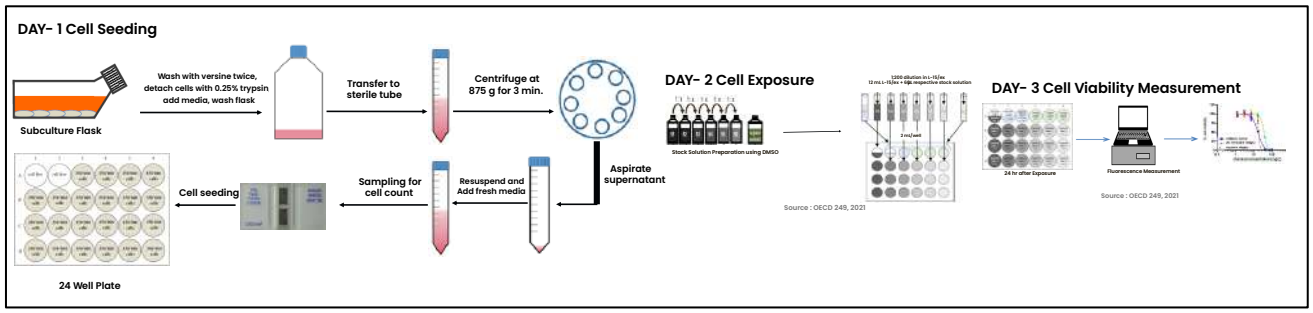
EXPERIMENTAL DESIGN

Acute Exposure

Groups | Replicates | Passage | Cells/well | Concentration selected | Observation | Endpoints

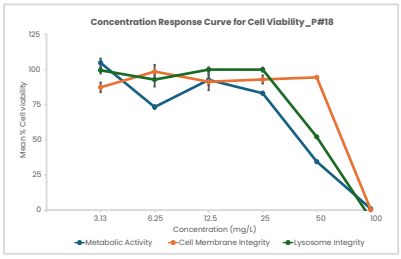
- Groups: 6
- Replicates: 3
- Passage: 18, 21, and 24
- Cells/well: 3,50,000
- Concentration selected: 3.13, 6.25, 12.5, 25.0, 50.0 and 100.0 mg DCA/L along with negative and solvent controls
- Observation: 24 h after exposure
- Endpoints: Evaluation of the median effective concentration (EC_{50})

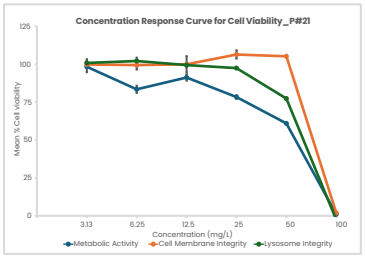
EXPERIMENTAL PROCEDURE

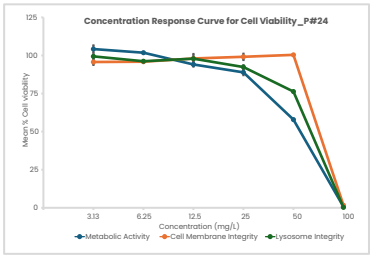


RESULTS

Fluorescence Dye	EC_{50} (mg/L) Passage			2½ SD EC_{50} (Range given in OECD 249)
	18	21	24	
Alamar Blue	31.6	40.6	45.4	28.4 - 58.9 mg/L
CFDA-AM	69.8	68.7	71.6	15.2 - 109.8 mg/L
Neutral Red	46.1	54.5	52.1	12.1 - 105.0 mg/L







CONCLUSION

The variation for cell viability of solvent control was lower than 10% than the negative control in terms of cell viability, which was within the range of natural background variability. The EC_{50} values of all the fluorescent indicator dyes are within the 2½ SD range in the OECD 239. Therefore, this study underlines the robustness of the RTgill-W1 cell line assay and its accurate performance at the **JAI RESEARCH FOUNDATION.**

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