

Use of the RTgill-W1 Cell Line Assay to Assess Acute Fish Toxicity



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Environmental Risk Assessment

Environmental risk assessment of chemicals is a critical step in ensuring their safe use & management. It plays a vital role in regulatory frameworks like Classification, Labelling and Packaging (CLP).

Aquatic species, especially fishes, serve as key indicators for water quality assessment and contamination due to their sensitivity to these substances.

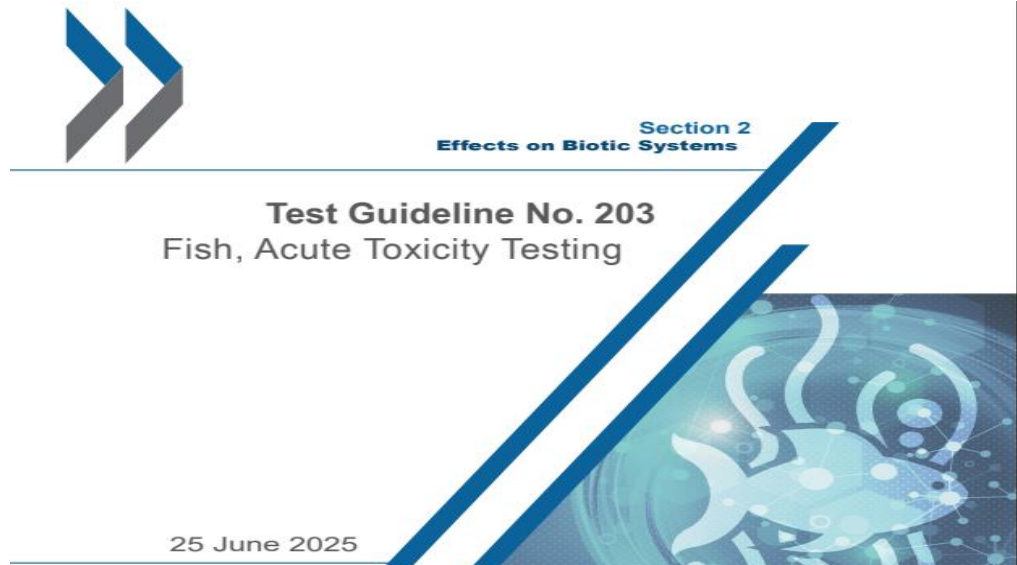
For Registration of a new conventional pesticide, regulatory frameworks prescribed by the Code of Federal Regulations (CFR), CIB&RC and other international guidelines may require acute toxicity data for one cold freshwater (e.g., rainbow trout), one warm freshwater (e.g., bluegill sunfish), and one saltwater fish (e.g., sheepshead minnow) species.

The traditional toxicology studies have relied heavily on *in vivo* testing with whole organisms. However, such approaches often require significant resources and raise ethical concerns over the use of live animals.

Fish cell lines have emerged as a New Approach Methodology (NAM) tool for environmental toxicology studies.

Alternatives to Fish Acute Toxicity Test

In vivo Toxicity Test



In vitro Toxicity Test



Topics Countries & regions P

OECD > Publications > Test No. 236: Fish Embryo Acute Toxicity (FET) Test

Test No. 236: Fish Embryo Acute Toxicity (FET) Test

Report




Topics Countries & regions

OECD > Publications > Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay

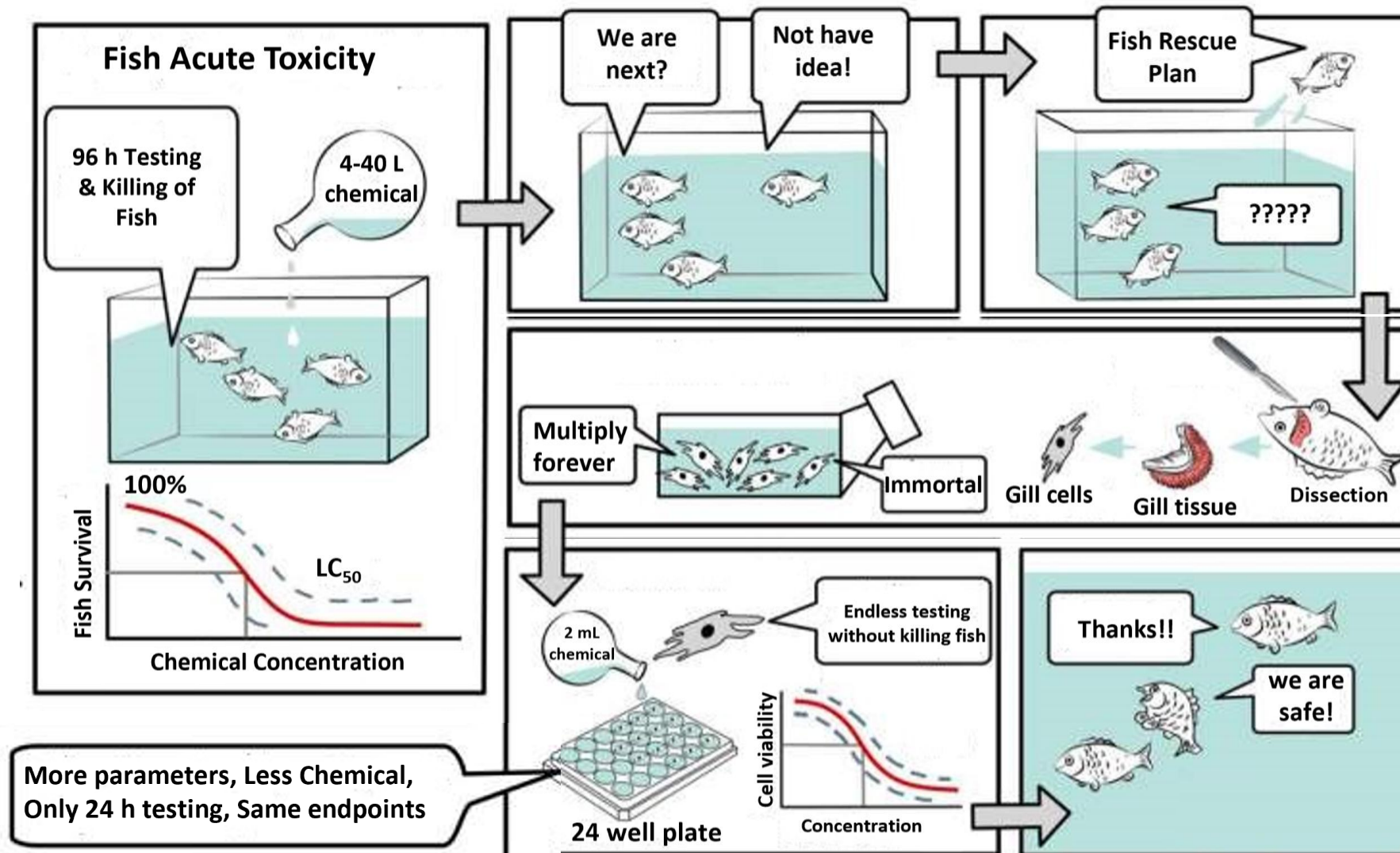
Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay

Report

More info 



Alternatives to Fish Acute Toxicity Test





Toxicity Test for Risk Assessment

OECD TG 210: Fish, Early-life Stage Toxicity Test

OECD TG 212: Fish, Short-term Toxicity Test on
Embryo and Sac-Fry Stages

OECD TG 215: Fish, Juvenile Growth Test

OECD TG 229: Fish Short Term Reproduction
Assay

OECD TG 234: Fish Sexual Development Test




Topics Countries & regions

OECD > Publications > OECD Guidelines for the Testing of Chemicals, Section 2

OECD Guidelines for the Testing of Chemicals, Section 2

Book series

More info 



Refinements to Fish Acute Toxicity Test



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In vitro Methods support 3Rs

OECD TG 319A: Determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP)

OECD TG 319B: Determination of *in vitro* intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)

OECD TG 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure



Topics Countries & re

OECD > Publications > OECD Guidelines for the Testing of Chemicals, Section 3

OECD Guidelines for the Testing of Chemicals, Section 3

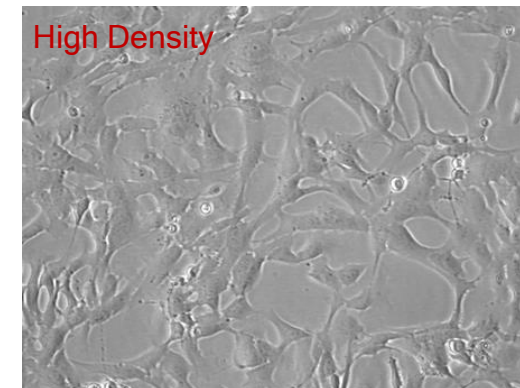
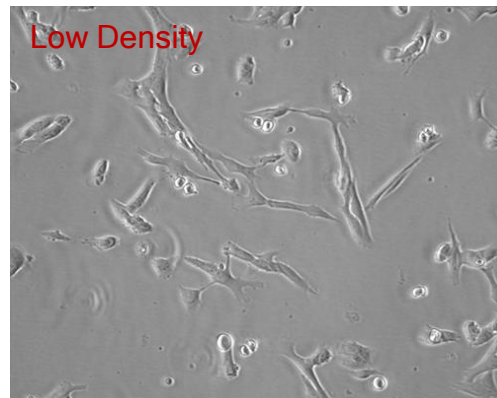
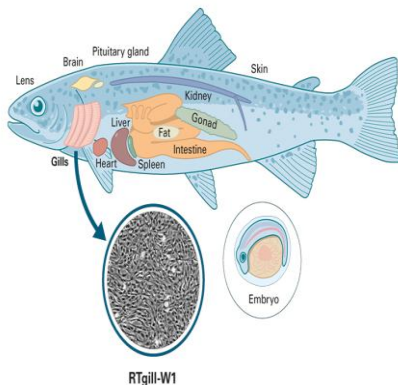
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Introduction (OECD TG 249)

- RT gill-W1 cell line was initiated from gill filaments of a healthy rainbow trout, one of the standard test species listed in OECD TG 203.
- The gills are unique organ involved in gas exchange, osmoregulation and other critical functions essential for survival of fish.
- The gills are the primary target and uptake sites of water contamination.
- RTgill-W1 cells grow adherent and are cultivated as monolayer cultures. The cell line is propagated in cell culture flasks at 19 ± 1 °C, without any additional CO₂ supply.
- At low density the cells have an irregular spindly shape, but with reaching confluence, the cells exhibit a polygonal shape and are tightly packed.





Application

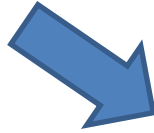
- The OECD 249 guideline supports the use of these tests to generate toxicity data. This data can be integrated with other approaches, such as Quantitative Structure-Activity Relationships (QSAR)—which predict chemical behavior based on molecular structure—and the Weight of Evidence (WoE) approach, which considers all available information, to provide a more comprehensive assessment of chemical toxicity.
- Its first application in the context of environmental risk assessment was for effluent testing, an application for which an International Standardization Organization guideline (ISO 21115; ISO 2019) was adopted in April 2019.
- These cells are ideal sentinel models for *in vitro* aquatic toxicology as well as model systems to study fish gill function and gill diseases.
- The cells have been used to understand mechanisms of toxicity, ranking the potencies of toxicants, and evaluating the toxicity of environmental samples.



Principle



RTgill-W1 cells in confluent monolayers in 24-well cell culture plates are exposed to the test chemical for 24 h in the dark

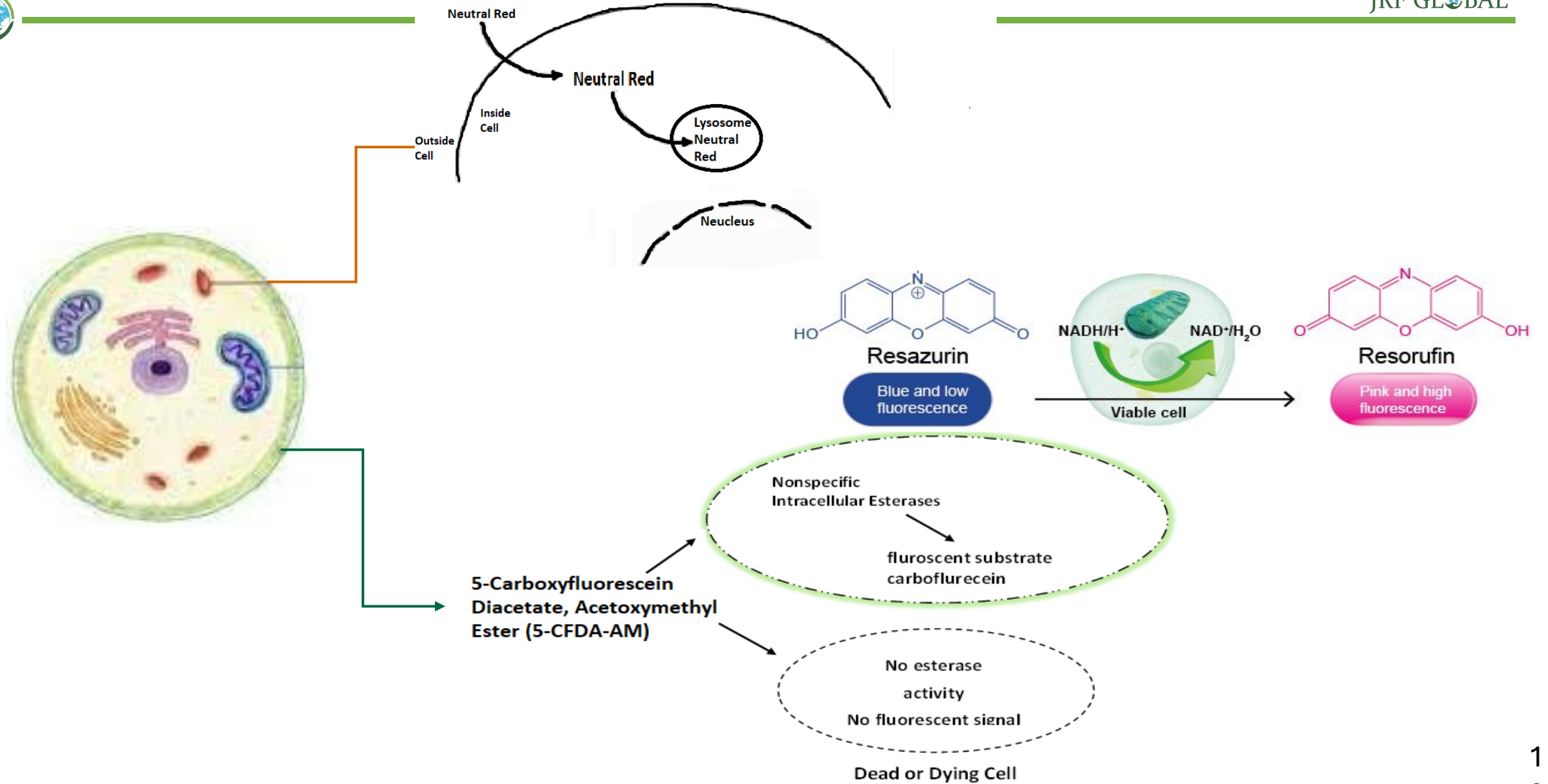


cell viability is assessed with a set of fluorescent cell viability indicator dyes



EC₅₀, LOEC/NOEC
(Where possible)

Principle (Continued)



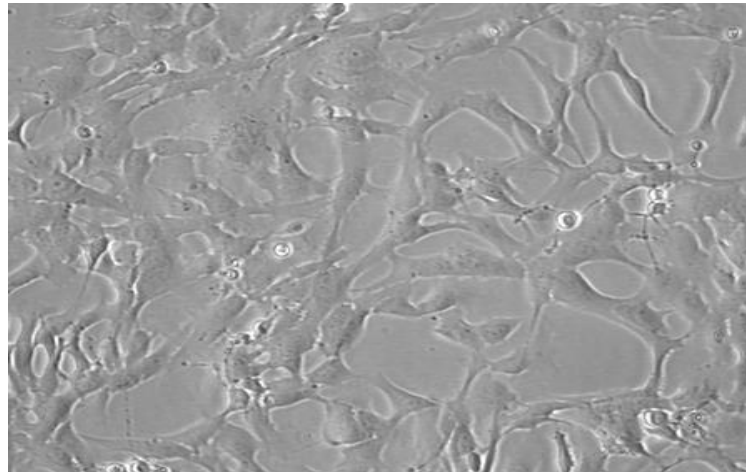


Test Chemical

- In the current trial, positive control substance, 3,4-dichloroaniline has been used.
- 3,4-Dichloroaniline is used as intermediate of pesticide, pharmaceutical and raw material for agriculture pesticides.
- It is easy to handle (intermediate water solubility) and is used as positive control in the zebrafish egg/embryo test (FET OECD TG 236; OECD 2013).

Test System

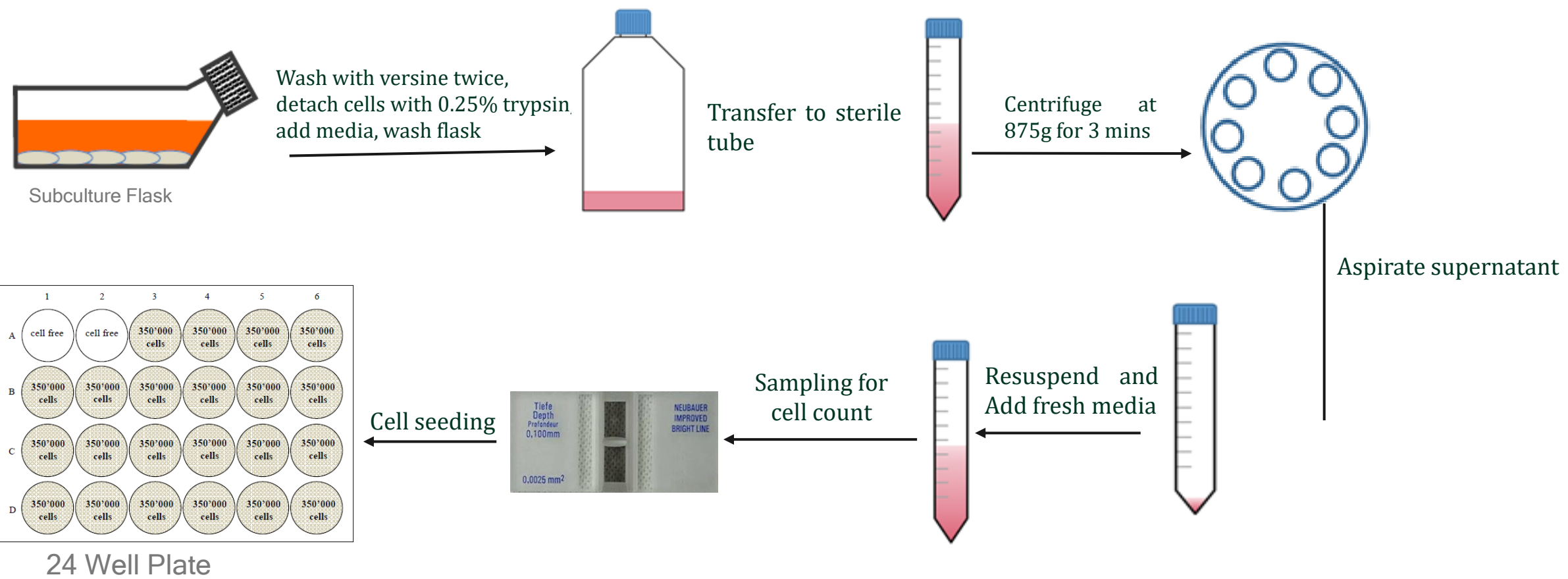
- **RTgill-W1 Cells**



Experimental Procedure



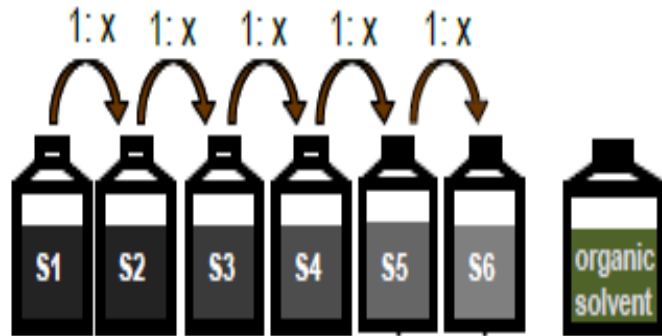
DAY- 1 Cell Seeding



Experimental Procedure (Continued)

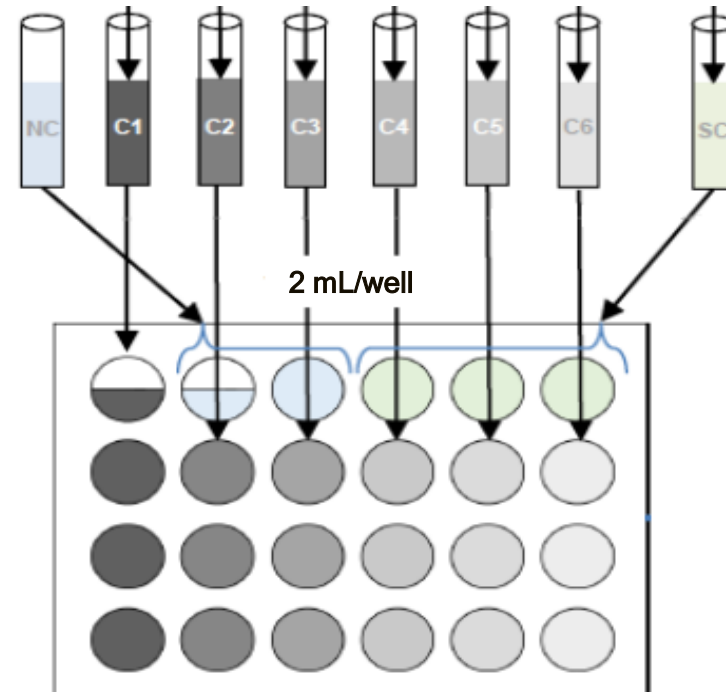


DAY- 2 Cell Exposure



Stock Solution Preparation using DMSO

1:200 dilution in L-15/ex
12 mL L-15/ex + 60 μ L respective stock solution



Fluorescence Measurement

DAY- 3 Cell Viability Measurement



Acute Exposure	
Groups	6
Replicates	3
Cells/well	3,50,000 cells
Concentration selected	3.13, 6.25, 12.5, 25.0, 50.0 and 100.0 mg/L along with Negative controls, Solvent Control
Observation interval	24 h after exposure
End points	Evaluate the median effective concentration (EC ₅₀)

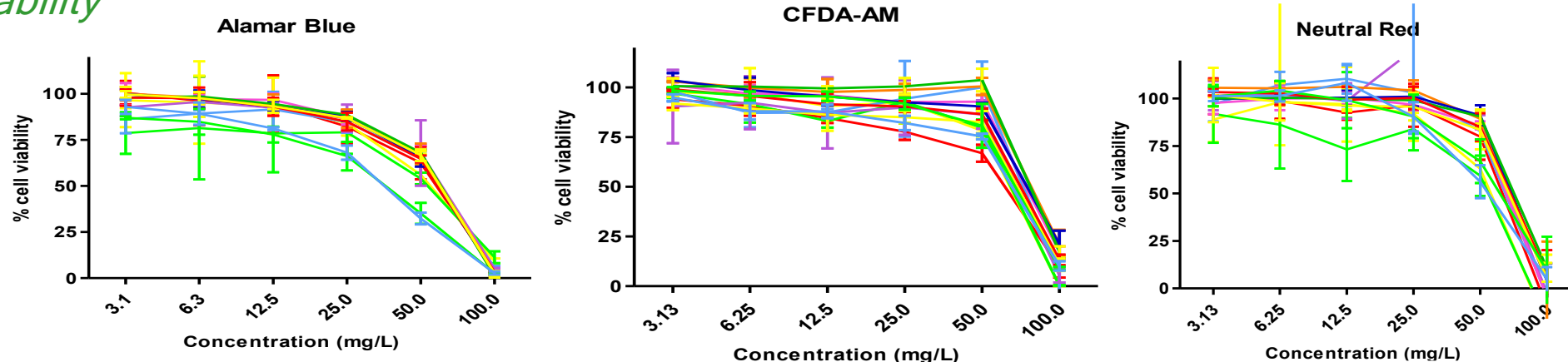
2½ SD EC ₅₀ value of Indicators for 3,5-Dichloroaniline	
Resazurin-based dye	28.4 - 58.9 mg/L
CFDA-AM (5-carboxyfluorescein diacetate acetoxy methyl ester)	15.2 - 109.8 mg/L
Neutral Red	12.1 - 105.0 mg/L



Result



% cell Viability



Trial	Alamar Blue			CFDA-AM			Neutral Red		
	EC ₅₀	95% Fiducial CI		EC ₅₀	95% Fiducial CI		EC ₅₀	95% Fiducial CI	
	mg/L	Lower	Upper	mg/L	Lower	Upper	mg/L	Lower	Upper
1	36.8	20.1	67.3	46.8	27.6	79.5	48.3	20.4	114.5
2	47.6	30.0	75.6	52.4	17.3	159.1	37.3	11.2	123.9
3	38.5	17.3	85.7	47.4	11.4	197.3	31.9	11.4	88.8
4	51.1	29.5	88.5	68.4	43.9	106.6	67.7	18.8	244.2
5	54.3	33.5	88.0	73.0	40.2	132.7	61.1	29.3	127.5
6	45.9	31.9	66.2	67.8	40.0	114.7	45.9	12.7	165.4
7	42.7	30.2	60.3	40.0	27.7	57.9	39.7	32.7	48.2
8	43.1	30.0	61.9	49.7	30.3	81.5	69.6	47.4	102.1
9	55.3	26.9	113.7	39.8	29.3	54.2	39.9	30.2	52.7
10	46.1	30.7	69.3	73.5	43.6	124.0	39.7	29.4	53.7
11	44.1	30.5	63.7	70.3	37.2	132.6	43.0	29.3	63.1
12	46.5	36.9	58.6	82.8	64.3	106.5	62.6	53.6	73.2
13	48.7	39.3	60.3	74.1	55.6	98.7	62.3	54.4	71.4

Conclusion



- The variation of raw fluorescence data of the two cell-free control wells was found to be below 20%.
- The cell viability of the solvent control was within the range of 10% compared to the negative control, which is within the range of natural background variability.
- The mean nominal EC_{50} values for the 3,4-DCA were found to be 36.8 – 55.3 mg/L, 39.8 – 82.8 mg/L, and 31.9 – 69.6 mg/L for Alamar Blue, CFDA-AM, and Neutral Red, respectively, which is within two-and-a-half standard deviations (SD) from the EC_{50} values recommended in OECD TG 249 for the test to be valid.
- This study, therefore, highlights the robustness of the RTgill-W1 cell line assay and its accurate performance, as carried out at Jai Research Foundation, to assess acute toxicity in fish for pesticides and other environmental chemicals.



References



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- OECD (2021), Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.



Predicting Fish Acute Toxicity Using a Fish Gill Cell Line-Based Toxicity Assay

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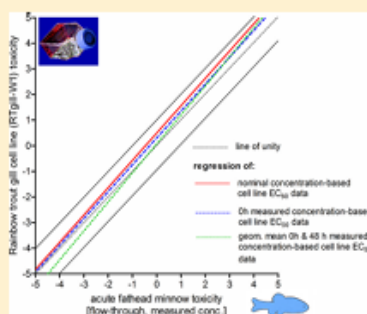
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S Supporting Information

ABSTRACT: The OECD test guideline 203 for determination of fish acute toxicity requires substantial numbers of fish and uses death as an apical end point. One potential alternative are fish cell lines; however, several studies indicated that these appear up to several orders of magnitude less sensitive than fish. We developed a fish gill cell line-based (RTgill-W1) assay, using several measures to improve sensitivity. The optimized assay was applied to determine the toxicity of 35 organic chemicals, having a wide range of toxicity to fish, mode of action and physicochemical properties. We found a very good agreement between *in vivo* and *in vitro* effective concentrations. For up to 73% of the tested compounds, the difference between the two approaches was less than 5-fold, covering baseline toxicants but as well compounds with presumed specific modes of action, including reactivity, inhibition of acetylcholine esterase or uncoupling of oxidative phosphorylation. Accounting for measured chemical concentrations eliminated two outliers, the hydrophobic 4-decylaniline and the volatile 2,3-dimethyl-1,3-butadiene, with an outlier being operationally defined as a substance showing a more than 10-fold difference between *in vivo*/*in vitro* effect concentrations. Few outliers remained. The most striking were allyl alcohol (2700-fold), which likely needs to be metabolically activated, and permethrin (190-fold) and lindane (63-fold), compounds acting, respectively, on sodium and chloride channels in the brain of fish. We discuss further developments of this assay and suggest its use beyond predicting acute toxicity to fish, for example, as part of adverse outcome pathways to replace, reduce, or refine chronic fish tests.



Environmental Science & Technology

Article

Table 1. EC₅₀ Values and Corresponding 95% Confidence Intervals for the End Point of Metabolic Activity Compared to LC₅₀ Data Obtained from the U.S. EPA fathead Minnow Database^a

chemical	abbreviation	based on nominal chemical concentrations		based on measured C _{0,t} concentrations		based on geometric mean concentrations (C _{0,t} and C _{24,t})		LC ₅₀ ^d (mg/L)
		EC ₅₀ (mg/L)	95% confidence interval	EC ₅₀ (mg/L)	95% confidence interval	EC ₅₀ (mg/L)	95% confidence interval	
ethanol	EtOH	30 250	15 330–59 700	no chemical analysis				14 200
2,2,2-trichloroethanol	TCE	123.8	87.5–175	106.5	75.3–150.6	102.1	72.75–143	298.1
diethylphthalate	DEP	63.8	53.3–76.4	69.0	56.1–84.9	63.9	52.4–77.9	32.1
di-n-butylphthalate	DBP	0.63	0.55–0.71	0.34	0.29–0.39	0.25	0.21–0.29	0.83
4-decylaniline	4DA	1.59	1.32–1.92	0.56	0.41–0.77	0.01	0.009–0.03	0.06
naphthalene	NAP	39.4	35.6–43.6	28.1	25.9–30.6	8.6	7.50–9.80	6.13
1,2-dichlorobenzene	DCB	31.9	27.1–37.5	26.9	22.9–31.6	7.14	5.87–8.68	9.49
dichloromethane	DCM	1918	1646–2234	1499	1291–1742	1036	979.5–1095	330.4
tetrachloroethylene	TeCE	97.4	88.7–106.9	32.4	30.2–34.7	7.42	7.25–7.60	13.4
1,2,4-trichlorobenzene	TCB	16.2	13.2–19.8	14.2	11.6–17.2	2.23	1.87–2.67	3.01
aniline	ANI	801.7	635.3–1012	442.2	337.0–580.4	461.7	360.1–592.0	134.6
4-chlorophenol	4CP	14.4	11.3–18.4	12.0	9.52–15.2	10.9	8.57–13.7	6.15
2,4,6-trichlorophenol	TCP	2.16	1.59–2.94	2.00	1.45–2.78	1.91	1.37–2.67	9.24
3,4-dichloroaniline	DCA	14.0	12.5–15.6	7.75	6.91–8.69	6.57	5.88–7.33	7.07
N-methylaniline	NMA	191.9	146.9–250.5	161.8	129.5–202.1	159.3	124.7–203.4	100.0
sodium dodecyl sulfate	SDS	2.13	1.93–2.35	no chemical analysis				6.76
allyl alcohol	AAL	836.1	642.1–1089	834.3	641.2–1086	812.3	622.2–1060	0.30
ethanal	ETH	32.8	26.5–40.7	no chemical analysis				30.5
acrolein	ACR	0.22	0.20–0.60	no chemical analysis				20.0
menadione	MED	0.37	0.32–0.44	0.36	0.31–0.43	0.12	0.10–0.14	0.11
2,3-dimethyl-1,3-butadiene	DMBD	188.0	175.1–201.8	82.2	77.8–86.8	15.2	13.9–16.6	6.83
dichlorophene	DCP	0.17	0.14–0.21	0.12	0.10–0.14	0.05	0.04–0.06	0.31
4-fluoroaniline	4FA	662.9	557.7–788.0	212.6	179.1–252.2	200.0	165.7–241.4	16.8
hexachlorophene	HCP	0.03	0.02–0.04	0.02	0.01–0.03	0.01	0.007–0.01	0.02
rotenone	ROT	1.0 × 10 ⁻⁴	0.8 × 10 ⁻⁴ – 1.3 × 10 ⁻⁴	no chemical analysis				0.005
2,4-dinitrophenol	DNP	0.54	0.47–0.61	0.76	0.66–0.88	0.77	0.68–0.87	13.3
pentachlorophenol	PCP	0.02	0.02–0.02	0.02	0.01–0.02	0.01	0.01–0.02	0.22
malathion	MAL	11.1	8.59–14.3	13.0	10.2–16.6	12.9	10.0–16.6	14.1
disulfoton	DIS	2.72	2.42–3.05	2.5	2.25–2.84	1.90	1.69–2.13	3.97
parathion ethyl	PAR	0.85	0.74–0.99	0.84	0.73–0.97	0.81	0.70–0.94	1.58 ^e
permethrin	PER	11.4	7.52–17.26	9.0	6.12–13.2	3.76	2.98–4.76	0.02
lindane	LIN	11.7 ^b	nd ^c	9.7 ^b	nd ^c	6.9 ^b	nd ^c	0.11 ^f
caffeine	CAF	2621	2336–2940	2726	2429–3061	2652	2357–2984	150.7
hexamethylenetetramine	HMT	15 050	12 470–18 170	13 850	11 550–16 620	13 870	11 550–16 660	49 741
2-aminoethanol	2AE	94.4	87.6–101.6	no chemical analysis				2069.7



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