Syngene

Putting Science to Work

Catalogue of Regulatory *In Vitro* and Genetic Toxicology Screening Assays

Index

Alternative Toxicology: *In Vitro* Tests

Genetic Toxicology Tests

Overview of Syngene's Safety Assessment Services

Syngene has a full service genetic toxicology department to support the genotoxic potential characterization of compounds from screening to IND submission. Our scientific staff and state-of-the-art facilities provide the most current and comprehensive genetic toxicology studies as per OECD guidelines. We have been executing genetic toxicology studies since 2010 and have an able and experienced team of toxicologists, pathologists and statisticians. In the last two years itself we have completed over 200 genetic toxicology studies for our partners.

In vitro toxicology is a key aspect of the discovery and predictive phase of product development. They can increasingly provide stand-alone data for specific endpoints without *in vivo* confirmatory studies. Also, *in vitro* methods developed are more predictive of the human response and have a quicker turnaround time. They act as the crossover points between drug discovery and drug development and are widely used for screening and ranking of chemicals by studying specific target to cell and tissue. In addition to the standard genotoxicity studies such as bacterial reverse mutation, micronucleus test and chromosomal aberration, we conduct in vitro cytotoxicity assay using neutral red uptake method, which is used to screen the cytotoxic potential of compounds. Recently we have also included *in vitro* phototoxicity test using Balb/c 3T3 method and in vitro skin irritation using Reconstructed Human Epidermis, skin corrosion using Reconstructed human epidermis and eye irritation using Reconstructed Human Cornea-like Epithelium test method.

ALTERNATIVE TOXICOLOGY: *IN VITRO* TESTS

IN VITRO TESTS	OECD GUIDELINE NUMBER
In Vitro Skin Corrosion Test : Reconstructed Human Epidermis (RHE)	OECD 431
In Vitro Skin Irritation Test : Reconstructed Human Epidermis (RHE)	OECD 439
In Vitro 3T3 NRU Phototoxicity Test: Balb/C	OECD 432
In Vitro 3T3 NRU Cytotoxicity Test : Balb/C	ISO 10993-5:2009
In Vitro Eye Irritation or Serious Eye Damage Test : Reconstructed human Cornea-like Epithelium (RhCE)	OECD 492
In Vitro Skin Absorption	OECD TG 428
In Chemico Skin Sensitisation	OECD TG 442C

In Vitro Skin Corrosion Test: Reconstructed Human Epidermis (RHE)

In Vitro Skin Corrosion Test is used for the hazard identification of those chemicals and mixtures capable of inducing skin corrosion (UN GHS Category 1), and in some cases to partially subcategorize corrosives into UN GHS Sub-Categories 1A or 1B and 1C

Test Model	Epi Derm, Mattek
Replicates	N=2 tissues per test condition
Assay Controls	Negative Control – Ultrapure WaterPositive Control – 8N KOH
Exposure Time	1 hour topical exposure to Epi derm50 µL or 25mg of test material per tissue
Test Item Quantity	0.5 ml or 500 mg
End Point	MTT Tissue Viability Assay
Data Delivery	% relative viability ± SD
Timeline	15 days

Acceptable OD value for Epi derm Model:

- Lower acceptance limit: ≥ 0.8
- Upper acceptance limit: ≤ 2.8

Mean tissue viability (%)	Data prediction
3 minutes < 50%	Corrosive (1A)
3 min ≥ 50% and1 hour < 15%	Corrosive (1B and 1C)
3 min ≥ 50% and1 hour ≥ 15%	Non-corrosive

In Vitro Skin Irritation Test: Reconstructed Human Epidermis (RHE)

In Vitro Skin Irritation Test is used for the hazard identification of irritant chemicals (substances and mixtures) in accordance with the UN Globally Harmonized System of Classification and Labelling (GHS) Category 2. Predict the potential of test compound to cause dermal irritation

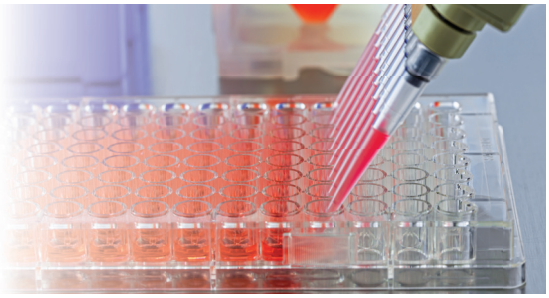
Test Model	Epi Derm, Mattek
Replicates	N=3 tissues per test condition
Assay Controls	Negative Control – Sterile Phosphate bufferPositive Control – 5% Sodium dodecyl Sulphate
Exposure Time	1 hour topical exposure to Epi derm30µl or 25mg of test material per tissue
Test Item Quantity	0.5 ml or 500 mg
End Point	MTT Tissue Viability Assay
Data Delivery	% relative viability ± SD
Timeline	10 days

Acceptable OD value for Epi derm Model:

- Lower acceptance limit: ≥ 0.8
- Upper acceptance limit: ≤ 2.8

Mean tissue viability (%)	Category	Data prediction
≤ 50%	Category 2	Irritant (I)
> 50%	No Category	Non Irritant (NI)

In Vitro 3T3 NRU Phototoxicity Test

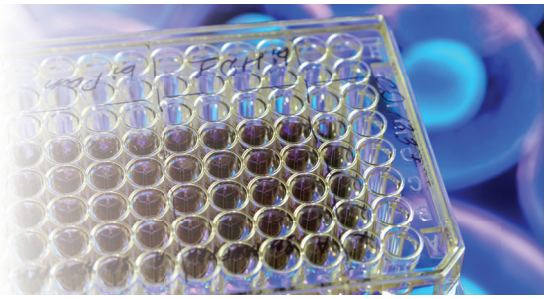


The *in vitro* 3T3 Neutral Red Uptake (NRU) phototoxicity test is used to identify the phototoxic potential of a test chemical activated by exposure to light. The test evaluates photo-cytotoxicity by the relative reduction in viability of cells exposed to the test chemical in the presence versus absence of light

Test Model	Balb/c 3T3 cells
Replicates	Six per group (Preliminary and Confirmatory study)
Assay Controls	Negative Control – Aqueous or organic solvents Positive Control – Chlorpromazine (CPZ) Eight different Test Item concentrations
Exposure Time	5 J/cm2 for 50 minutes
Test Item Quantity	1.0 gram
End Point	The ability of viable cells to incorporate and bind neutral red (NR) in the presence and absence of light
Data Delivery	IC50 Calculation and Photo-Irritation Factor
Timeline	3 - 4 Weeks

In Vitro 3T3 NRU Cytotoxicity Test

The Neutral red uptake (NRU) cytotoxicity test is based on the ability of viable cells to incorporate and bind neutral red (NR).



Test Model	Balb/c 3T3 cells
Replicates	Six per group (Preliminary and Confirmatory study)
Assay Controls	Negative Control – Aqueous or Organic Solvents Positive Control – Sodium Lauryl Sulfate Eight different Test Item concentrations
Exposure Time	44 - 48 hours
Test Item Quantity	1.0 gram
End Point	The ability of viable cells to incorporate and bind neutral red (NR)
Data Delivery	% of viability of cells
Timeline	3 - 4 Weeks

In Vitro Eye Irritation or Serious Eye Damage Test : Reconstructed human Cornea-like Epithelium (RhCE)

In Vitro eye irritation is used to evaluate the eye hazard potential of a test chemical based on its ability to induce cytotoxicity in a RhCE tissue construct, as measured by the tetrazolium dye.

Test Model	Epi Ocular Tissues
Replicates	N=2 tissues per test condition
Assay Controls	Negative Control – Ultrapure Water Positive Control – Methyl acetate
Exposure Time	6 hours (±0.25 h) - Solids 30 min (±2 min) - Liquids 50 µL or 50mg of test material per tissue
Test Item Quantity	0.5 ml or 500 mg
End Point	MTT Tissue Viability Assay
Data Delivery	% relative viability ± SD
Timeline	15 days

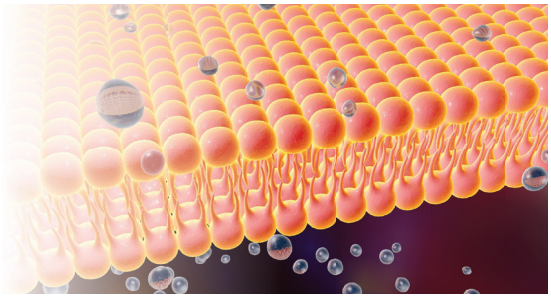
Acceptable OD value for Epi Ocular Model:

- Lower acceptance limit: ≥ 0.8
- Upper acceptance limit: ≤ 2.8

Mean tissue viability (%)	Data prediction
≤ 60%	Irritant (I)
> 60%	Non Irritant (NI)

In Vitro Skin Absorption

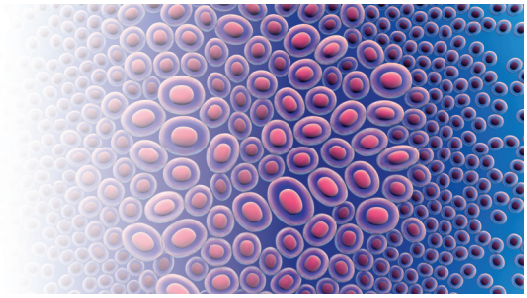
In Vitro Skin Absorption study is used to evaluate the *topical* skin penetration of the test item, which is applied to the surface of a skin sample separating the two chambers of a diffusion cell apparatus. The chemical remains on the skin for a specified time &conditions before removal by an appropriate cleansing procedure.



Test Model	Human Epiderm and Artificial Synthetic Membrane
Replicates	N=4, Number of Donors will be decided based on client requirement
Assay Controls	1-5 mg/cm2 of the skin for solid test item 10 µL/cm2 of skin for liquid test item
Exposure Time	HPLC Method Development and Validation of Test compound 24 h at 32° C ±1° C, at 400 rpm Samples 1.0 mL of the receptor fluid collection points (1, 2, 4, 8, and 24 h post-dosing) for analysis
Test Item Quantity	1000 mg
End Point	The skin values were expressed as mean ± SD for each compartment. The mean recovery (%) of test item (donor chamber, skin wash, skin homogenate and receptor fluid) should be 100 ±10 %
Data Delivery	Mean recovery (%) of test item
Timeline	6 - 7 weeks

In Chemico Skin Sensitisation

This study evaluates the test item with Direct Peptide Reactive Assay (DPRA) *In Chemico* method measured by reverse HPLC, which quantifies Cysteine and Lysine peptide percent depletion values. The calculation is done with reference to prediction model (Mean of Cysteine and Lysine % depletion), which allows assigning the test item to one of four reactivity classes used to support the discrimination between sensitisers and non-sensitisers.



Test Model	Peptides (Cysteine and Lysine)
Replicates	N= 4
Assay Controls	HPLC Method Development and Validation of Test compound Reference Control A (verify suitability of the reverse control A), B (To verify stability of reference controls during beginning and end of the analysis) Reference control C (to verify the solvent used to dissolve the test item does not impact the percent peptide depletion) Co-elution controls
Exposure Time	Cysteine or Lysine containing peptide following 24 hours incubation with the test items at 22.5°C
Test Item Quantity	1000 mg
End Point	Mean of Cysteine and Lysine % depletion
Data Delivery	Classify as Sensitizers and Non-Sensitizer as per % depletion of Cysteine and Lysine
Timeline	6 - 7 weeks

GENETIC TOXICOLOGY TESTS

GENETIC TOXICOLOGY TESTS	OECD GUIDELINE NUMBER
Bacterial Reverse Mutation Test - AMES Test	OECD TG 471
In Vitro Chromosomal Aberration Test	OECD TG 473
In Vitro Micronucleus Test	OECD TG 487
Mammalian Erythrocyte Micronucleus Test	OECD TG 474
Mammalian Bone Marrow Chromosome Aberration Test	OECD TG 475
HPRT cell gene Mutation Test	OECD TG 476

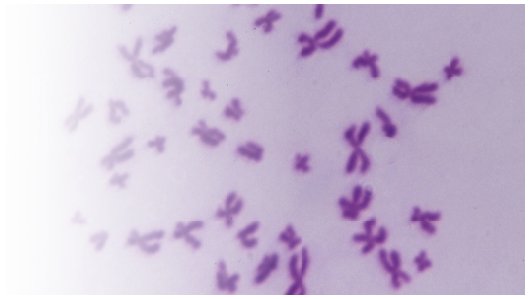
Bacterial Reverse Mutation Test - AMES Test

Syngene helps toxicologists reduce AMES test turnaround time from 3 days to same-day results & reports. Using sensitive GLP and 21 CFR compliant Sorcerer colony counter with Cyto Study manager software, we merged sophisticated image processing and analysis with sensitive cameras and motorization for quick, precise bacterial and mammalian colony counts.

Test Model	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA97, TA97a, TA102, and E.coli WP2 UvrA
Assay Controls	Negative control: Aqueous and organic solvents Positive control: Strain specific positive control
Exposure Time	48 - 72 hours
Test Item Quantity	2.0 grams
End Point	Growth of revertant colonies
Data Delivery	Mean number of relevant colonies per plate digitized using colony counter with Cyto study manager 2-fold increase with vehicle control (TA 98, TA 100, TA97, TA97a, TA102, and WP2 UvrA) 3-fold increase with vehicle control (TA 1535 and TA 1537)
Timeline	4 - 5 Weeks

In Vitro Chromosomal Aberration Test

The purpose of the *in vitro* chromosome aberration test is to identify agents that cause structural chromosomeaberrations in cultured mammalian somatic cells. Metaphase cells are analysed microscopically for the presence of chromosome aberrations.



Test Model	Chinese Hamster Ovary (CHO), Human peripheral blood lymphocytes
Assay Controls	Negative Control – Aqueous and Organic solvents Positive Control – Ethyl Methane Sulphonate and Cyclophosphamide monohydrate
Exposure Time	Short Duration – (With and Without Metabolic Activation, 3 - 6 hrs) Prolonged duration treatment: (Without Metabolic Activation, 20 - 21 hrs)
Test Item Concentration	Should not be greater than 10mM, 2 mg/mL or 2 µL/mL
End Point	Scoring of 300 metaphases to identify aberrations
Data Delivery	Percent Chromosomal aberrations and type of chromosomal aberrations.Mitotic Index
Timeline	10 - 12 Weeks

In Vitro Micronucleus Test

The *in vitro* micronucleus test is a genotoxicity test for the detection of micronuclei in the cytoplasm of interphase cells.

Test Model	Chinese Hamster Ovary (CHO), Human peripheral blood lymphocytes
Assay Controls	Negative Control – Aqueous and Organic solvents Positive Control – Methyl Methane Sulphonate/Ethyl Methane Sulphonate, Cyclophosphamide monohydrate and Vinblastine
Exposure Time	Short Duration – (With and Without Metabolic Activation, 3 - 6 hrs) Prolonged duration treatment: (Without Metabolic Activation, 20 - 21 hrs)
Test Item Concentration	10 mM, 2 mg/mL or 2 µl/mL
End Point	Micronucleus evaluation in 2000 binucleate cells
Data Delivery	Percentage of micronuclei and Replication Index
Timeline	10 - 12 Weeks

Mammalian Erythrocyte Micronucleus Test

The mammalian *in vivo* micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts, by analysis of erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals, usually rodents (mice or rats).

Test Model	Mice, Rats
Assay Controls	Vehicle Control: Appropriate vehicle control Positive Control: Cyclophosphamide monohydrate
Exposure Time	24 and 48 hours. Integrated with repeat dose toxicology studies
Test Item Quantity	Top dose concentration – Quantity may vary 2000 mg/kg – 13.2 grams
End Point	Bone marrow or Blood Micronucleus (4000 Polychromatic erythrocytes/Reticulocytes)
Data Delivery	Percentage of micronuclei compared with vehicle control
Timeline	11 - 12 Weeks

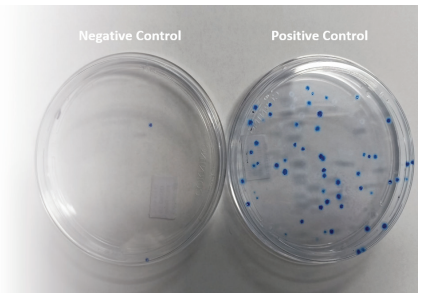
Mammalian Bone Marrow Chromosome Aberration Test

The mammalian *in vivo* chromosome aberration test is used for the detection of structural chromosome aberrations induced by test compounds in bone marrow cells of animals, usually rodents (rats and mice).

Test Model	Mice, Rats
Assay Controls	Vehicle Control: Appropriate vehicle control Positive Control: Cyclophosphamide monohydrate
Exposure Time	24 Hours and 48 hours
Test Item Quantity	Top dose concentration – Quantity may vary 2000 mg/kg – 13.2 grams
End Point	Bone marrow chromosomal aberrations in 200 metaphase
Data Delivery	Percentage of chromosomal aberration compared with vehicle control Mitotic Index
Timeline	11 - 12 Weeks

HPRT Cell Gene Mutation Test

The *in vitro* mammalian cell gene mutation test can be used to detect gene mutations induced by chemical substances.



Test Model	CHO K1
Assay Controls	Vehicle Control: Aqueous or organic solvents Positive Controls: Ethyl Methane sulphonate 7,12-Dimethylbenzanthracene
Exposure Time	3 - 6 hours 20 - 22 hours
Test Item Concentration	10 mM, 2 mg/mL or 2 µL/mL
End Point	Number of cells plated in selective and non-selective medium.
Data Delivery	Relative Survival and Mutant Frequency.
Timeline	10 - 11 Weeks

OVERVIEW OF SYNGENE'S SAFETY ASSESSMENT SERVICES

Advance your tox studies in a fast, robust and reliable manner

Syngene offers a full range of *In vivo* and *In vitro* toxicology services for comprehensive nonclinical development of pharmaceuticals

Exploratory studies	GLP studies	Phase I	Phase II	Phase III	Specialty studies
Pharmacokinetics	Analytical and bioanalytical studies to support safety tox program	Repeat dose studies & Genetic toxicology	Sub-chronic toxicity study		Impurity qualification
(MTD/Dose Escalation/Short-term repeat dose toxicology)	Repeat Dose Toxicology (2 Species)				Dermal Tox- Rat & Rabbit
Genotoxicity screening *(In vitro)*	Genotoxicity *(In vitro/ In vivo)*	Repeat dose studies & Genetic toxicology	Reproductive Toxicology (Male fertility/ Pre and Postnatal development)		Intravenous Infusion - Rat
Safety Pharmacology: CNS, Respiratory, CV	Toxicokinetics		Chronic Toxicology		Local tolerance/ irritation – Rats & Rabbits
	Core Battery Safety Pharmacology				Guniea Pig skin sensitization/ LLNA Mice
	Reproductive Toxicology (Seg I, Seg II & Seg IIII)				Medical Device Testing – ISO10993
					In vitro Cytotoxicity
					In vitro Skin Irritation
					In vitro Phototoxicity
					In vitro skin senstization

Our fully accredited, state-of-the-art vivarium has everything needed for executing PK & toxicology for critical path studies

70,000
Sq. ft. lab

AAALAC
accredited

OECD GLP
certified

CPCSEA
(Govt. of India) registered

IAEC & IBSC
approval for protocol

Exploratory/ regulatory
toxicology

AAALAC: *Association for Assessment and Accreditation of Laboratory Animal Care* | ***CPCSEA:*** *Committee for the Purpose of Control and Supervision of Experiments on Animals* | ***GLP:*** *Good laboratory practice* | ***IAEC:*** *Institute Animal Ethics Committee* | ***IBSC:*** *Institutional Bio-Safety Committee*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About Syngene

Syngene Syngene International Ltd. (BSE: 539268, NSE: SYNGENE, ISIN: INE398R01022) is an integrated research, development, and manufacturing services company serving the global pharmaceutical, biotechnology, nutrition, animal health, consumer goods, and specialty chemical sectors. Syngene's more than 6000 scientists offer both skills and the capacity to deliver great science, robust data security, and quality manufacturing, at speed, to improve time-to-market and lower the cost of innovation. With a combination of dedicated research facilities for Amgen, Baxter, and Bristol-Myers Squibb as well as 2.2 Mn sq. ft of specialist discovery, development and manufacturing facilities, Syngene works with biotech companies pursuing leading-edge science as well as multinationals, including GSK, Zoetis and Merck KGaA.

For more details, visit www.syngeneintl.com or write to us at bdc@syngeneintl.com

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