"GenPharmTox is one of the few CROs who can guarantee the success of our project within the given time framework and on the terms agreed."
Dr. Michael Runkel, Apogepha GmbH

3

Toxicology
in vitro / in vivo

3 Toxicology *in vitro* / *in vivo*

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In vitro Services

3.1. Phototoxicity Test

The standard *in vitro* 3T3 NRU Phototoxicity Test is performed according to OECD draft guideline 432. Screening and ranking study designs are also available.

3.1.1. Standard Phototoxicity Test (OECD 432, Draft)

The phototoxic potential of the test item is investigated by comparison of the cytotoxicity (EC_{50} values) under illumination and in the dark.

Quantity of test item required: 400 mg
Turnaround time of draft report: 20 working days

Study Design

- range finding pre-test
- Balb/c 3T3 cells
- 8 dose levels
- treatment: 1 h in the dark, followed by 50 min. at 1.7 mW/cm²
- negative control
- positive control
- measurement of Neutral Red uptake

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3.2. Cytotoxicity / Hepatotoxicity Test

Cytotoxicity is a well established and easily accessable endpoint to get first information on the general / acute toxic potential of a test item. In order to further investigate the possible involvement of metabolism and the mechanism of toxicity different test systems and / or endpoints should be investigated.

Test Systems

- low metabolic competence: V79 cells (parental)
- high metabolic competence: primary hepatocytes (see chapter 2.5.1.)
- specific metabolic competence: V79 Cell Battery™ (see chapter 4.)

Three different Endpoints: for cytotoxicity can be measured:

- mitochondrial activity: MTT-Assay
- membrane integrity: Neutral Red Uptake
- total protein content: Sulforhodamine B-Assay

Please inquire for further test systems and endpoints.

3.2.1. Screening Cytotoxicity / Hepatotoxicity Test

The relative cell viability upon incubation with test item compared to the solvent control is determined (single point).

Quantity of test item required: 1 mg (pre-weighted)
Turnaround time of draft report: 10 working days

Study Design

- V79 cells, primary hepatocytes, etc.
- one concentration of test item
- four replicates
- one incubation time
- positive control
- negative control

3.2.2. Standard Cytotoxicity / Hepatotoxicity Test

The relative cell viability upon incubation with test item compared to the solvent control is determined (dose-effect curve and calculation of the EC_{50}).

Quantity of test item required: 15 mg
Turnaround time of draft report: 10 working days

Study Design

- V79 cells, primary hepatocytes, etc.
- ten concentrations of test item
- eight replicates
- incubation time: 4 to 72 h
- positive control
- negative control

3.2.3. Advanced Cytotoxicity Test (V79 Cell Battery™)

The relative cell viability upon incubation with test item compared to the solvent control is determined (dose-effect curve and calculation of the EC_{50}).

Quantity of test item required: 15 mg
Turnaround time of draft report: 10 working days

Study Design

- recombinant V79 cells expressing phase I and / or phase II enzymes (see chapter 4.: V79 Cell Battery™)
- internal metabolic activation
- ten concentrations of test item
- eight replicates
- incubation time: 24 – 72 h
- positive control
- negative control

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3.3. AMES Test

The standard Ames test (bacterial reverse mutation test) is performed according to OECD guideline 471. Screening and ranking study designs are also available.

Please inquire for Ames II screening assay and Ames Prival modification.

3.3.1. Screening AMES Test

The mutagenic potential of the test item in a bacterial test system is investigated (single dose, 1 *S. typhimurium* strain).

Quantity of test item required: 20 mg
Turnaround time of draft report: 10 working days

Study Design

- 1 *S. typhimurium* strain: e.g. TA97a, TA98, TA100, TA102, TA1535, TA1537, TA1538, or *E. coli* WP2 uvrA
- 1 experiment w and w/o S9 (rat), preincubation assay
- 1 dose level
- 3 plates per dose level
- 4 plates as viability control
- 3 plates per negative (vehicle) control
- 3 plates per positive control
- report including documentation of signs for insolubility or cytotoxicity and number of revertant colonies

3.3.2. Ranking AMES Test

The mutagenic potential of the test item in a bacterial test system is investigated (dose-effect curve, 2 *S. typhimurium* strains).

Quantity of test item required: 70 mg
Turnaround time of draft report: 10 working days

Study design

- 2 *S. typhimurium* strains: e.g. TA97a, TA98, TA100, TA102, TA1535, TA1537, TA1538, and/or *E. coli* WP2 uvrA
- 1 experiment w and w/o S9 (rat), preincubation assay
- 5 dose levels
- 3 plates per dose level
- 4 plates as viability control
- 3 plates per negative (vehicle) control
- 3 plates per positive control
- report including documentation of signs for insolubility or cytotoxicity, and number of revertant colonies

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3.3.3. Standard AMES Test (OECD 471)

The mutagenic potential of the test item in a bacterial test system is investigated (dose-effect curve, 5 *S. typhimurium* strains). The study design and documentation / reporting can be adopted to the requirements of the Japanese regulatory authorities .

Quantity of test item required: 2 mg
Turnaround time of draft report: 20 working days

Study design

- 5 *S. typhimurium* strains: TA97a, TA98, TA100, TA102, TA1535, and/or TA1537, TA1538, E. *coli* WP2 uvrA
- cytotoxicity and solubility pre tests
- w and w/o S9 (rat), preincubation assay
- 5 dose levels
- 3 plates per dose level
- 4 plates as viability control
- 3 plates per negative control
- 3 plates per positive control

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3.4. HPRT Test

The H(G)PRT test (hypoxanthine guanine phosphoribosyl transferase test; mutagenicity assay *in vitro*: mammalian cell gene mutation test) detects mutagenic effects of the test item in mammalian cells. The test is performed according to OECD guideline 476. Screening and ranking study designs are also available.

3.4.1. Standard HPRT Test (OECD 476)

Quantity of test item required: 3 g
Turnaround time of draft report: 35 working days

Study Design

- cytotoxicity and range finding pre-test
- V79 cells
- four dose levels
- two replicate cultures per dose level
- three subcultures per replicate
- treatment: 4 h or 24 h w/o metabolic activation, 4 h with metabolic activation
- plating efficiency control
- negative control
- positive control

3.4.2. Advanced HPRT Test (V79 Cell Battery™, OECD 476)

Quantity of test item required: 2 g
Turnaround time of draft report: 35 working days

Study Design

- cytotoxicity and range finding pre-test
- recombinant V79 cells expressing phase I and / or phase II enzymes (see chapter 4.: V79 Cell Battery™)
- internal metabolic activation
- four dose levels
- two replicate cultures per dose level
- three subcultures per replicate
- treatment: 24 to 72 hours
- plating efficiency control
- negative control
- positive control

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3.5. Mouse Lymphoma Assay

The mouse lymphoma assay (mutagenicity assay *in vitro*: mammalian cell gene mutation test) detects mutagenic and clastogenic effects of the test item in mammalian cells. The assay is performed according to the OECD guideline 476. Screening and ranking study designs are also available.

Quantity of test item required: 1 g
Turnaround time of draft report: 20 working days

Study Design

- cytotoxicity and range finding pre-test
- L5178Y/TK$^{+/-}$ mouse lymphoma cells, heterozygous at the thymidine kinase (TK) locus
- two replicate cultures per dose level
- three subcultures per replicate
- treatment: 3 h w and w/o metabolic activation, 24 h w/o metabolic activation
- 4 dose levels
- negative (vehicle) control
- positive control
- plating efficiency control
- selection period: 10 – 14 days

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3.6. Comet Assay

The comet assay (single-cell gel electrophoresis assay, SCGE) measures DNA strand breaks at the level of single cells from any tissue or cell culture.
The assay is performed according to "Guidelines for *in vitro* and *in vivo* Genetic Toxicology Testing: Single Cell Gel/Comet Assay" by R. Tice *et al.* (2000). Screening and ranking study designs are also available.

3.6.1. Standard Comet Assay (Guideline by R. Tice *et al.*, 2000)

Quantity of test item required: 500 g
Turnaround time of draft report: 15 working days

Study Design

- cytotoxicity and range finding pre-test
- V79 cells or others
- duplicates
- 5 dose levels
- treatment: 3 – 6 h w and w/o metabolic activation, 24 h w/o metabolic activation
- negative (vehicle) control
- positive control
- microscopic analysis: 100 cells per duplicate

3.6.2. Advanced Comet Assay (V79 Cell Battery™, Guideline by R. Tice *et al.*, 2000)

Quantity of test item required: 500 mg
Turnaround time of draft report: 5 working days

Study Design

- recombinant V79 cells expressing phase I and / or phase II enzymes (see chapter 4.: V79 Cell Battery™)
- internal metabolic activation
- duplicates
- 5 dose levels
- treatment: 24 – 72 h
- negative (vehicle) control
- positive control
- microscopic analysis: 100 cells per duplicate

3.7. Micronucleus Test

The micronucleus test *in vitro* detects clastogenic effects of the test item in mammalian cells. The test is performed according to the OECD draft guideline by J. M. Parry. Screening and ranking study designs are also available.

3.7.1. Standard Micronucleus Test (OECD Draft Guideline by J. M. Parry)

Quantity of test item required: 2 g
Turnaround time of draft report: 20 working days

Study Design

- cytotoxicity and range finding pre-test
- V79 cells
- duplicates
- 3 dose levels
- with and without rat S9
- incubation time: 3 h w and w/o S9, 24 h w/o S9
- negative control
- positive control
- microscopic analysis: 1000 cells per duplicate
- detailed study report

3.7.2. Advanced Micronucleus Test (V79 Cell Battery™, OECD Draft Guideline by J. M. Parry)

Quantity of test item required: 2 g
Turnaround time of draft report: 20 working days

Study Design

- cytotoxicity and range finding pre-test
- recombinant V79 cells expressing phase I and / or phase II enzymes (see chapter 4.: V79 Cell Battery™)
- internal metabolic activation
- duplicates
- 3 dose levels
- incubation time: 24 – 72 h
- negative control
- positive control
- microscopic analysis: 1000 cells per duplicate

3.8. Chromosome Aberration Test

The chromosome aberration test (mutagenicity: *in vitro* mammalian cytogenetic test) is performed according to OECD guideline 473. Screening and ranking study designs are also available.

3.8.1. Standard Chromosome Aberration Test (OECD 473)

Quantity of test item required: 3 g
Turnaround time of draft report: 30 working days

Study Design

- cytotoxicity and range finding pre-test
- V79 cells
- 3 dose levels
- minimum of two replicates per dose level
- with and without rat S9
- incubation time: 3 h w and w/o S9, 24 h w/o S9
- negative control
- positive control
- evaluation of 200 metaphases per concentration

3.8.2. Advanced Chromosome Aberration Test (V79 Cell Battery™, OECD 473)

Quantity of test item required: 2 g
Turnaround time of draft report: 30 working days

Study Design

- cytotoxicity and range finding pre-test
- recombinant V79 cells expressing phase I and / or phase II enzymes (see chapter 4.:V79 Cell Battery™)
- internal metabolic activation
- 3 dose levels
- minimum of two replicates per dose level
- incubation time: 24 – 72 h
- negative control
- positive control
- evaluation of 200 metaphases per concentration

In vivo Services

3.1. General Considerations

3.1.1. Animal Welfare

It is the overall goal and dedication of GenPharmTox and its founders to replace, reduce, and refine animal experiments whenever possible.

Accordingly, GenPharmTox offers a broad range of well established and cutting-edge innovative *in vitro* assays for preclinical drug development as well as safety evaluation of chemicals. By the many successful projects performed with our clients we have built an exceptionally strong reputation in the area of *in vitro* testing.

The *in vivo* studies offered in co-operation with partners are planned and performed on the basis of sound science and in accordance with animal welfare legislation as well as other relevant regulatory requirements, e. g. OECD- and FDA-guidelines.

3.1.2. Homogeneity and Stability

For each substance tested in *in vivo* studies we recommend performing homogeneity and stability testing including analytical method validation / evaluation in the vehicle used.

3.1.3. Co-operations

The *in vivo* toxicological services are offered in co-operation with ProTox.

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3.2. Acute to Chronic Toxicity

- Single Dose Acute Oral Toxicity / Rodent (OECD 423)
- Single Dose Acute Dermal Toxicity / Rodent (OECD 402)
- Dermal Irritation / Rabbit (OECD 404)
- Eye Irritation / Rabbit (OECD 405)
- Sensitization Magnusson & Kligman (M&K) / Guinea Pig (OECD 406)
- Sensitization Bühler / Guinea Pig (OECD 406)
- Local Lymph Node Assay (LLNA) / Mouse (OECD 429)
- Local Tolerance / Rabbit (EMEA)
- 28-Days Repeated Dose Subacute Oral Toxicity / Rat (OECD 407)
- 28-Days Repeated Dose Subacute Dermal Toxicity / Rat (OECD 410)
- 90-Days Repeated Dose Subchronic Oral Toxicity / Rodent (OECD 408)
- 90-Days Repeated Dose Subchronic Oral Toxicity / Non-Rodent (OECD 409)
- 90-Days Repeated Dose Subchronic Dermal Toxicity / Rodent (OECD 408)
- 6-Months Repeated Dose Oral Toxicity / Rodent (EMEA)

3.3. Genotoxicity

- Chromosome Aberration *in vivo* / Mouse (OECD 473)
- Micronucleus *in vivo* / Mouse (OECD 475)

3.4. Carcinogenicity

- Combined Chronic Toxicity & Carcinogenicity / Rodent
- Dietary Carcinogenicity / Rat (OECD 541)
- Dietary Carcinogenicity / Mouse (EMEA)

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3.5. Reproduction Toxicity

- Prenatal Development Toxicity: Rat or Rabbit (OECD 414)
- Embryotoxicity (EMEA)
- Reproduction Toxicity: One Generation (OECD 415)
- Reproduction Toxicity: Two Generation (OECD 416)

3.6. Aquatic Toxicology

- Acute Toxicity / Fish (OECD 203)
- Acute Toxicity / Daphnia (OECD 202)
- Embryotoxicity / Fish (OECD draft)
- Early Life Stage / Fish (OECD 210)
- Prolonged Toxicity / Fish (OECD 204)
- Growth Inhibition / Alga (OECD 201)
- Respiration Inhibition / Activated Sludge (OECD 209)
- Juvenile Growth Test / Fish (OECD 215)
- 21-Days Reproduction Test / Daphnia (OECD 211)

3.7. Biological Degradation / Bioaccumulation

- Manometric Respirometry Test (OECD 301F)
- Closed Bottle Test (OECD 301D)
- CO_2 Evolution Test (OECD 301B)
- DOC - Die Away Test (OECD 301A)
- Zahn-Wellens / EMPA Test (OECD 302B)
- Toxicity / Earthworm (OECD 207)
- Bioaccumulation / Fish (OECD 305)
- Adsorption Coefficient (HPLC-Screening) (OECD 121)

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3.8. Avian Toxicity

- Acute Toxicity / Bobwhite or Japanese Quail or Mallard Duck (OPPTS850.2100)
- Dietary Toxicity / Bobwhite or Japanese Quail or Mallard Duck (OPPTS850.2200)
- 7-Weeks Reproduction: Range Finding / Bobwhite Quail
- 20-Weeks Reproduction Toxicity / Bobwhite Quail (OPPTS850.2300)
- 6-Months Reproduction Toxicity / Japanese Quail (OECD Draft)
- Palatibility / Japanese Quail

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