



Intelligence for life
GenPharmTox



SERVICES

ADME *in vitro* / *in vivo*

Toxicology *in vitro* / *in vivo*

V79 Cell Battery™

Analytical Services

Service Packages

**PLUS PARTNERED
SERVICES INSIDE !**



First Class Service from Target to Man



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Our Business
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in vitro / *in vivo*
 Toxicology
in vitro / *in vivo*
 V79
 Cell Battery™
 Analytical
 Services
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 Services

Our Business
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Our Business

“We set high standards in the collaboration with our partners. The expertise and personal commitment of GenPharmTox have convinced us and they have fully met our expectations.”

Dr. Henning Heinemann, Research Manager Solvay Pharmaceuticals, Hannover

Our Business



I.1. Our Mission

I.2. Our Expertise

I.3. Your Benefit

I.4. Our GLP Certificate

I.1. Our Mission

GenPharmTox stands for quality and safety in risk assessments for drugs and chemicals. As a professional service provider for the international pharmaceutical, chemical and biotechnology industries, we meet your demands and those of the market. Our customers are well known multinational corporations as well as small innovative biotechnology companies.

Mutual trust and your direct involvement in the process assures our joint success. Our highly qualified and motivated employees are aware of their responsibilities and ensure that you are delivered a quality service within the agreed timelines.

I.2. Our Expertise



On the basis of predictive *in vitro* test strategies, we succeed in advancing and optimising your development of drugs and the assessment of chemicals. Our employees accompany you through the preclinical development of your substances from screening through to approval. Top quality calls for a high degree of expertise and personal commitment, as a consequence we stay in close dialogue with you to facilitate the realisation of optimum solutions for you.

I.3. Your Benefit

Individual customer benefit in the preclinical fields is of prime importance to us. Through continuous communication and intensive collaboration with our customers and partners, we ensure that customers receive the optimum solutions to their problems.

Quality, safety, personal attention, and individual solutions are the key aspects of the collaboration with our customers.

I.4. Our GLP Certificate


**BAYERISCHES LANDESAMT
FÜR ARBEITSSCHUTZ,
ARBEITSMEDIZIN UND SICHERHEITSTECHNIK**
Pfarrstraße 3 · 80538 München · Telefon (089) 21 84-0


GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in: Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

Prüfeinrichtung/Test facility Prüfstandort/Test site

GenPharmTox BioTech AG
Fraunhoferstr. 9
82152 Martinsried
(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)


3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften
(in vitro)


Datum der Inspektion/Date of Inspection
(Tag,Monst.,Jahr/day,month,year)
06.03.2002

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht. The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können. Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

München, 11.04.2002


Dr. Deimer
Präsident



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ADME *in vitro / in vivo*

“Individual expertise in finding solutions at the preclinical stage and a high level of reliability are strengths we particularly appreciate in GenPharmTox for a partnership-based collaboration.”

Dr. Michael Gassen, 4SC AG



In vitro Services

2.1. Absorption

2.1.1. Caco-2 Permeability Assay

2.2. Metabolic Stability

2.2.1. Drug Discovery Screen

2.2.2. Drug Development Assay

2.3. CYP Profiling

2.3.1. Human Recombinant Enzymes

2.3.2. Chemical Inhibition

2.3.3. Correlation Analysis

2.4. Enzyme Kinetics

2.5. Species Comparison and Metabolite Profiling

2.5.1. Available Species, Strains and Test Systems

2.5.2. Study Outline

2.6. CYP Inhibition

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2.6.2. Determination of the IC₅₀

2.6.3. Determination of the K_i

2.7. CYP Induction

In vivo Services

2.1. General Considerations

2.2. Pharmacokinetics

In vitro Services

2.1. Absorption

2.1.1. Caco-2 Permeability Assay

Caco-2 cells are a well-established *in vitro* model to predict intestinal absorption of drugs and chemicals in humans. Plated Caco-2 cells on a membrane support allow the study of drug transport from the apical side to the basolateral side (A to B transport) as well as from the basolateral side to the apical side (B to A transport). The apical and basolateral sides of Caco-2 cells represent the luminal and blood sides, respectively, of the gastrointestinal tract *in vivo*. In addition, the transport kinetics can be investigated.

The apparent permeability (P_{app}) of the test item is calculated and compared with that of reference compounds of known permeability.

Quantity of test item required: 15 mg

Turnaround time of draft report: 20 working days

Transport

- triplicates
- Caco-2 cells (monolayer in Transwell® plates)
- one concentration of test item (50 µM)
- one time point (120 min)

Kinetics

- triplicates
- Caco-2 cells (monolayer in Transwell® plates)
- one to four concentrations of test item (12.5, 25, 50, 100 µM)
- one to six time points (0, 15, 30, 60, 90, 120 min)
- positive controls

Controls

- propranolol (high permeability, transcellular transport)
- hydrocortisone (moderate permeability, transcellular transport)
- ranitidine (low permeability, paracellular transport)
- vinblastine inhibited by verapamil (P-glycoprotein mediated transport)
- measurement of the transepithelial electrical resistance (TEER) to check for membrane integrity

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection: detection of parent compound

2.2. Metabolic Stability

The metabolic stability of the test item is determined using liver microsomes or primary hepatocytes of different species. The metabolic stability is also determined during “Metabolite Profiling and Species Comparison”.

2.2.1. Metabolic Stability as Drug Discovery Screen

The “percentage metabolism” of the test item is determined.

Quantity of test item required: 1 mg (pre-weighted)

Turnaround time of draft report: 5 working days

Study Design

- duplicates
- 0.5 mg/ml of human and rat liver microsomes or 0.5 x 10⁶ hepatocytes/ml
Please inquire for further species.
- one concentration of test item (10 µM)
- one time point (60 min)
- positive control: formation of 7-hydroxycoumarin
- negative control: zero time point or no NADPH

Analytics

- LC-MS/MS detection (loss of parent compound)

2.2.2. Metabolic Stability as Drug Development Assay

The “percentage metabolism” and the Cl_{int} and t_{1/2} of the test item are determined.

Quantity of test item required: 5 mg (pre-weighted)

Turnaround time of draft report: 20 working days

Study Design

- range finding (optional)
- triplicates
- one concentration of human and rat liver microsomes or primary hepatocytes
Please inquire other species.
- one concentration of test item
- five time points
- positive control: formation of 7-hydroxycoumarin
- negative control: zero time point or no NADPH

Analytics

- optimised LC-MS/MS detection
(loss of parent compound, metabolite profiling possible)

2.3. CYP Profiling

Identification of CYP isoforms involved in the metabolism of the test item *in vitro*.

2.3.1. Human Recombinant Enzymes

The “percentage metabolism” of the test item with respect to different human recombinant CYP isoenzymes is determined

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

Study Design

- 9 CYP isoenzymes
1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4
Please inquire for further CYP-isoforms.
- triplicates
- one concentration of CYP
- one concentration of test item
- one time point
- positive control: CYP marker reaction
- negative control: non-expressing control

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection:
loss of parent compound and/or formation of selected metabolites and/or identification of major oxidative metabolites by mass relative to the parent compound.

2.3.2. Chemical Inhibition

The inhibitory effect of different specific CYP inhibitors on the metabolism of the test item in pooled human liver microsomes is investigated.

Quantity of test item required: 15 mg (pre-weighted)
Turnaround time of draft report: 15 working days

Study Design

- range finding (optional)
- triplicates
- one concentration of pooled human liver microsomes
- one concentration of test item
- one time point
- one chemical inhibitor per CYP isoenzyme
(one to three concentrations)
- positive control: incubations without inhibitor
- negative control: zero time point and/or no NADPH

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection:
loss of parent compound and/or formation of selected metabolites and/or identification of major oxidative metabolites by mass relative to the parent compound

2.3.3. Correlation Analysis

The CYP isoenzymes involved in the *in vitro* metabolism of the test item are identified by correlation analysis using individual human liver microsomes. The inter-individual rate of metabolism of the test item is compared to the variation in the rate of a set of CYP marker reactions. The respective correlation coefficients are determined.

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

Study Design

- range finding (optional)
- triplicates
- 16 individual human liver microsomal samples (one concentration)
- one time point
- positive control: CYP marker reaction
- negative control: zero time point and/or no NADPH

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection: loss of parent compound and/or formation of selected metabolites and/or identification of major oxidative metabolites by mass relative to the parent compound

2.4. Enzyme Kinetics

The apparent kinetic constants (K_M , V_{max} , Cl_{int}) are determined using microsomes, primary hepatocytes or recombinant enzymes.

Quantity of test item required: 5 mg
Turnaround time of draft report: 20 working days

Range Finding

- single determinations
- three concentrations of test system (e.g. 0.5, 1, 1.5 mg microsomal protein/ml)
- three concentrations of test item (e.g. 1, 10, 100 μ M)
- three time points (e.g. 15, 30, 60 min)
- positive control: enzyme marker reaction (e.g. formation of 7-hydroxycoumarin)
- negative control: zero time point and no NADPH

Study Design

- triplicates
- one concentration of test system
- seven to ten concentrations of test item
- one time point
- positive control: enzyme marker reaction
- negative control: zero time point

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection: detection of loss of parent compound and/or formation of selected metabolites, quantitative

Comment

If no comparable data are available, a range-finding pretest is pre-requisite for the determination of the enzyme kinetics in order to ensure sufficient metabolism of the test item as well as linear response with time and test system concentration. Further, a reference metabolite should be available for calibration and calculation of the V_{max} and Cl_{int} . Alternatively, radiolabelled test item can be used. Indirect calculation by the loss of parent compound is usually limited by insufficient metabolism rates at high test item concentrations.

2.5. Species Comparison and Metabolite Profiling

The metabolic stability and metabolite profile of the test item is investigated and compared with respect to different species *in vitro*. The data obtained provide valuable information for species selection in toxicological studies *in vivo*.

2.5.1. Available Species, Strains and Test Systems

Species	Liver Microsomes	Primary Hepatocytes	
		cyropreserved	freshly isolated
Human	individual donor or pooled (male, female, mixed)	individual donor or pooled (male, female, mixed)	individual donor (male, female)
Rat	Sprague Dawley Fischer 344 Wistar IGS Sprague Dawley (male, female, mixed)	Sprague Dawley Wistar (male, female, mixed)	Sprague Dawley Fischer 344 IGS Sprague Dawley (male, female, mixed)
Dog	Beagle Dog (male, female, mixed)	Beagle Dog (male, female, mixed)	Beagle Dog (male, female)
Guinea Pig	Hartley Albino (male)	please inquire	please inquire
Rabbit	New Zealand (male)	New Zealand (male, female, mixed)	please inquire
Mouse	CD1 B6C3F1 (male) (male)	CD1	please inquire
Monkey	Rhesus Rhesus Cynomolgus (male, female, mixed)	Cynomolgus Cynomolgus (male, female, mixed)	(male, female)
Hamster	Golden Syrian (male)	please inquire	please inquire
Pig	please inquire (male)	Gottingen Minipig	individual animal

Please inquire for other species and tissues, gender, or induced animal products.

2.5.2. Study Outline

Quantity of test item required: 10 mg / species
Turnaround time of draft report: 20 – 30 working days

Study Design

- range finding (optional)
- triplicates
- one concentration of test system
- one concentration of test item
- one to six time points
- positive control:
formation of 7-hydroxycoumarin and/or
selected phase I and/or phase II marker reactions
- negative control:
zero time point and/or no NADPH
- TI blank (no test item)
- TS blank (not test system)
- TITS blank (no test item, no test system)

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection:
loss of parent compound and/or formation of selected
metabolites and/or identification of major phase I oxidative
metabolites and/or major phase II conjugates by mass relative
to the parent compound

2.6. CYP Inhibition

The inhibition of CYP isoenzymes is one of the key aspects in drug-drug interaction.

2.6.1. Drug Discovery Screen

The percentage inhibition of different CYP marker reaction activities by the test item is determined.

Quantity of test item required: 1 mg (pre-weighted)
Turnaround time of draft report: 15 working

Study Design

- multiple CYP isoforms (microsomes or human recombinant enzymes)
- triplicates
- one concentration of CYP
- 50 µM of test item
- 30 min
- negative controls: CYP marker reactions:
 - 1A1/2 (7-ethoxyresorufin O-deethylation)
 - 2A6 (coumarin 7-hydroxylation)
 - 2B6 (S-mephenytoin N-demethylation)
 - 2C8 (paclitaxel 6α-hydroxylation)
 - 2C9 (diclofenac 4'-hydroxylation)
 - 2C19 (S-mephenytoin 4'-hydroxylation)
 - 2D6 (bufuralol -hydroxylation)
 - 2E1 (chloroxazone 6-hydroxylation)
 - 3A4/5 (testosterone 6β-hydroxylation)
 - 4A11 (lauric acid-hydroxylation)
 - 11B1 (11-deoxycortisol 11β-hydroxylation)
 - 11B2 (deoxycorticosterone -hydroxylation)

- negative controls: CYP reference inhibitors:
 - 1A1/2 (furaflavine)
 - 2A6 (8-methoxypsoralene)
 - 2B6 (triethylenethiophosphoramidate)
 - 2C8 (ketoconazole)
 - 2C9 (sulfaphenazole)
 - 2C19 (omeprazole)
 - 2D6 (quinidine)
 - 2E1 (diethyldithiocarbamate)
 - 3A4/5 (ketoconazole)
 - 4A11 (10-(imidazolyl)-decanoic acid)
 - 11B1 (metyrapone)
 - 11B2 (ketoconazole)

Analytics

- detection of metabolite formed in the marker reaction

Please inquire for further phase I and II enzyme marker reactions and reference inhibitors.

2.6.2. Determination of the IC₅₀

The IC₅₀ of the test item for a specific CYP marker reaction or drug – drug interaction is determined.

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

Study Design

- one CYP isoform (microsomes or human recombinant enzymes)
- triplicates
- one concentration of CYP
- one concentration of marker substrate
- seven to ten concentrations of test item
- one time point
- negative controls: CYP marker reactions:
 - 1A1/2 (7-ethoxyresorufin O-deethylation)
 - 2A6 (coumarin 7-hydroxylation)
 - 2B6 (S-mephenytoin N-demethylation)
 - 2C8 (paclitaxel 6α-hydroxylation)
 - 2C9 (diclofenac 4'-hydroxylation)
 - 2C19 (S-mephenytoin 4'-hydroxylation)
 - 2D6 (bufuralol-hydroxylation)
 - 2E1 (chloroxazone 6-hydroxylation)
 - 3A4/5 (testosterone 6β-hydroxylation)
 - 4A11 (lauric acid-hydroxylation)
 - 11B1 (11-deoxycortisol 11β-hydroxylation)
 - 11B2 (deoxycorticosterone -hydroxylation)
- positive controls: CYP reference inhibitors:
 - 1A1/2 (furaflavine)
 - 2A6 (8-methoxypsoralene)
 - 2B6 (triethylenethiophosphoramidate)
 - 2C8 (ketoconazole)
 - 2C9 (sulfaphenazole)
 - 2C19 (omeprazole)
 - 2D6 (quinidine)
 - 2E1 (diethyldithiocarbamate)
 - 3A4/5 (ketoconazole)
 - 4A11 (10-(imidazolyl)-decanoic acid)
 - 11B1 (metyrapone)
 - 11B2 (ketoconazole)

Analytics

- detection of metabolite formed in the marker reaction

Please inquire for further phase I and II enzyme marker reactions and reference inhibitors.

2.6.3. Determination of the K_i

The K_i of the test item for a specific CYP isoform marker reaction or drug – drug interaction is determined.

Quantity of test item required: 20 mg

Turnaround time of draft report: 20 working

Study Design

- one CYP isoform (microsomes or human recombinant enzymes)
- triplicates
- one concentration of CYP
- three to six concentrations of marker substrate
- three to six concentrations of test item
- one time point
- negative controls: CYP marker reactions:
 - 1A1/2 (7-ethoxyresorufin O-deethylation)
 - 2A6 (coumarin 7-hydroxylation)
 - 2B6 (S-mephenytoin N-demethylation)
 - 2C8 (paclitaxel 6a-hydroxylation)
 - 2C9 (diclofenac 4'-hydroxylation)
 - 2C19 (S-mephenytoin 4'-hydroxylation)
 - 2D6 (bufuralol-hydroxylation)
 - 2E1 (chloroxazone 6-hydroxylation)
 - 3A4/5 (testosterone 6β-hydroxylation)
 - 4A11 (lauric acid-hydroxylation)
 - 11B1 (11-deoxycortisol 11β-hydroxylation)
 - 11B2 (deoxycorticosterone –hydroxylation)
- positive controls: CYP reference inhibitors:
 - 1A1/2 (furafylline)
 - 2A6 (8-methoxypsoralene)
 - 2B6 (triethylenethiophosphoramidate)
 - 2C8 (ketoconazole)
 - 2C9 (sulfaphenazole)
 - 2C19 (omeprazole)
 - 2D6 (quinidine)
 - 2E1 (diethyldithiocarbamate)
 - 3A4/5 (ketoconazole)
 - 4A11 (10-(imidazolyl)-decanoic acid)
 - 11B1 (metyrapone)
 - 11B2 (ketoconazole)

Analytics

- detection of metabolite formed in the marker reaction

Please inquire for further phase I and II enzyme marker reactions and reference inhibitors.

2.7. CYP Induction

The induction of CYP isoenzymes is one of the key aspects in drug-drug interaction.

The induction of CYP isoenzymes by the test item is investigated using plated cryopreserved or freshly isolated human primary hepatocytes. The relative fold induction compared to the solvent control is calculated on the level of mRNA, protein, and/or enzymatic activity and compared with that of known reference inducers.

Quantity of test item required: 10 mg

Turnaround time of draft report: 10 – 40 working

Study Design

- cytotoxicity pre-test (optional)
- triplicates
- 48 h recovery phase
- 24 to 72 h induction phase
- one concentration of primary hepatocytes (freshly isolated or cryopreserved)
- one to three concentrations of test item
- negative control: solvent control
- positive control: CYP reference inducers:
 - 1A2 (omeprazole)
 - 2A6 (pyrazole)
 - 2B6 (phenobarbital, rifampicin)
 - 2C9 (phenobarbital)
 - 2C19 (phenobarbital, rifampicin)
 - 2E1 (ethanol)
 - 3A4 (rifampicin)

Analytics

- enzymatic activity (CYP marker reactions)
- mRNA level (real-time PCR)
- CYP protein content (Western blotting)

Please inquire for further phase I and II enzyme marker reactions and reference inducers.

In vivo Services

2.1. General Considerations

2.1.1. 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.

2.1.2. 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.

2.2. Pharmacokinetics

Currently, GenPharmTox is establishing a new co-operation to provide *in vivo* pharmacokinetic studies.

Please inquire for further information.

“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*



In vitro Services

3.1. Phototoxicity Test

3.2. Cytotoxicity / Hepatotoxicity Test

3.3. AMES Test

3.4. HPRT Test

3.5. Mouse Lymphoma Assay

3.6. Comet Assay

3.7. Micronucleus Test

3.8. Chromosome Aberration Test

In vivo Services

3.1. General Considerations

3.2. Acute to Chronic Toxicity

3.3. Genotoxicity

3.4. Carcinogenicity

3.5. Reproduction Toxicity

3.6. Aquatic Toxicology

3.7. Biological Degradation / Bioaccumulation

3.8. Avian Toxicity

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 (EC₅₀ 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

3.2. Cytotoxicity / Hepatotoxicity Test

Cytotoxicity is a well established and easily accessible 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 EC₅₀).

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 EC₅₀).

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

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

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

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

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

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.

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)

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)
- CO₂ 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)

3.8. Avian Toxicity

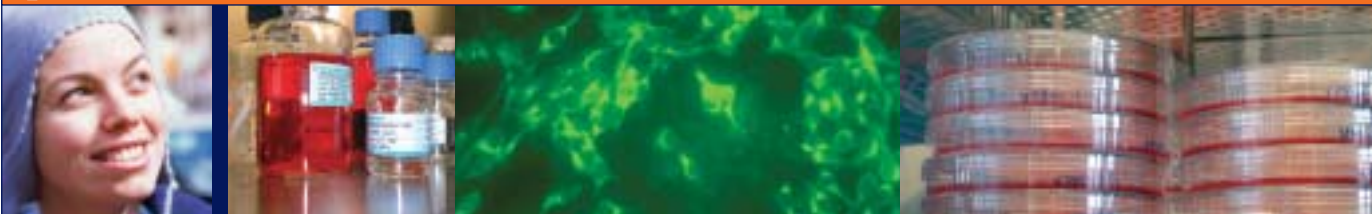
- Acute Toxicity / Bobwhite or Japanese Quail or Mallard Duck (OPPTS850.2100)
- Dietary Toxicity / Bobwhite or Japanese Quail or Mallard Duck (OPPTS850.2200)
- 7-Weeks Reproduction: Range Finding / Bobwhite Quail
- 20-Weeks Reproduction Toxicity / Bobwhite Quail (OPPTS850.2300)
- 6-Months Reproduction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

V79 Cell Battery™

4

“Professor Doehmer is one of the first toxicologists to apply the use of mammalian cell lines expressing human recombinant P450-isoenzymes in the prediction of drug metabolism and toxicity. This success has led to the confirmation of the heterogeneity of drug metabolism in different species and is an alternative to animal models for the evaluation of toxicity.”

Taken from the DFG expert report (August 30, 2000)



4.1. V79 Cell Battery™ for Phase I Enzymes

4.2. V79 Cell Battery™ for Phase II Enzymes

- 4.2.1. Glutathion S-Transferases (GST)
- 4.2.2. N-Acetyl-Transferases (NAT)
- 4.2.3. UDP-Glucuronosyl-Transferases (UGT)
- 4.2.4. Sulfo-Transferases (SULT)

GenPharmTox BioTech AG offers many of its contract services using the unique V79 Cell Battery™.

The V79 Cell Battery™ consists of a panel of recombinant V79 cell lines expressing a broad range of phase I and / or phase II enzymes relevant in the metabolism of xenobiotics.

GenPharmTox BioTech AG also provides cell homogenate and subcellular fractions of these recombinant V79 cell lines.

V79 cells provide unique biological properties:

- stable diploide karyotype
- cloning efficiency >90%
- doubling time <12 h
- stable morphology, robust culture
- no CYP background activity
- proven in toxicology since the 1960's
- recommended by the OECD guidelines

These properties make the V79 Cell Battery™ an unique *in vitro* test system:

- **Humanized:** Humanized systems guarantee high predictivity of *in vitro* results for the human situation.
- **Integrated:** Integrated system with identical location of metabolism and toxicological endpoint.
- **Specific:** Specificity enables to clarify metabolic pathways as well as mechanisms in toxicology.
- **Standardized:** Standardized systems guarantee high reproducibility.
- **Easy to use:** Stable and reproducible homogenous systems.
- **Economic:** Excellent cost / benefit ratio due to improved data quality with high predictive value for the human situation.

"A number of developments, including the construction of genetically engineered cell lines expressing specific activating enzymes, may provide the potential for endogenous activation. The choice of the cell lines used should be scientifically justified (e.g. by the relevance of the cytochrome P450 isoenzymes for the metabolism of the test substance)."

OECD recommendations

4.1. V79 Cell Battery™ for Phase I Enzymes

Human CYP:

Liver

- hCYP 1A2
- hCYP 2A6
- hCYP 2B6
- hCYP 2C8
- hCYP 2C9
- hCYP 2D6
- hCYP 2E1
- hCYP 3A4
- hCYP 3A5

Lung

- hCYP 1A1
- hCYP 1A2
- hCYP 2E1
- hCYP 2F1
- hCYP 3A4
- hCYP 4B1

Adrenal Gland

- hCYP 11B1
- hCYP 11B2

Polymorphic Variants

- hCYP 2C9*1 (wt), *2, *3
- hCYP*2D6*1 (wt), *2, *9, *10, *17

Rat CYP:

- rCYP 1A1
- rCYP 1A2
- rCYP 1B1
- rCYP 2B1
- rCYP 2E1

Fish CYP:

- fCYP 1A1

Mouse CYP:

- mCYP 1A1
- mCYP 1B1

4.2. V79 Cell Battery™ for Phase II Enzymes

4.2.1. Glutathion S-Transferases (GST)

Human GST:

- hGST A1
- hGST A2
- hGST M1a
- hGST P1
- hGST T1
- hGST T2

Combinations of Human CYP and Human GST

- h1A1 + hGST M1a
- h1A1 + hGST P1

Murine GST

- mGST M1
- mGST A4

Rat GST

- rGST 55

Combinations of Rat CYP and Human or Murine GST

- rCYP 1A1 + hGST P1
- rCYP 1A1 + hGST M1a
- rCYP 2B1 + mGST Yc2
- rCYP 2B1 + mGST Ya1
- rCYP 1A1 + mGST P1
- hGST T2

4.2.2. N-Acetyl-Transferases (NAT)

Human NAT2

- hNAT2*4 (wt)
- hNAT2*5B
- hNAT2*6A
- hNAT2*13

Combinations of Human CYP and Human NAT2

- hCYP1A2 + hNAT2*4 (wt)
- hCYP1A2 + hNAT2*5B
- hCYP1A2 + hNAT2*6A
- hCYP1A2 + hNAT2*13

4.2.3. UDP-Glucuronosyl-Transferases (UGT)

Please contact us for details.

4.2.4. Sulfo-Transferases (SULT)

Please contact us for details.

“As an innovative company we need a partner who fits in with our philosophy. GenPharmTox is a reliable, competent and brisk partner in realisation of our new development projects.”

Dr. Thomas Kronbach, elbion AG

5

Analytical Services



5.1. Analytical Services

5.2. Instrumentation

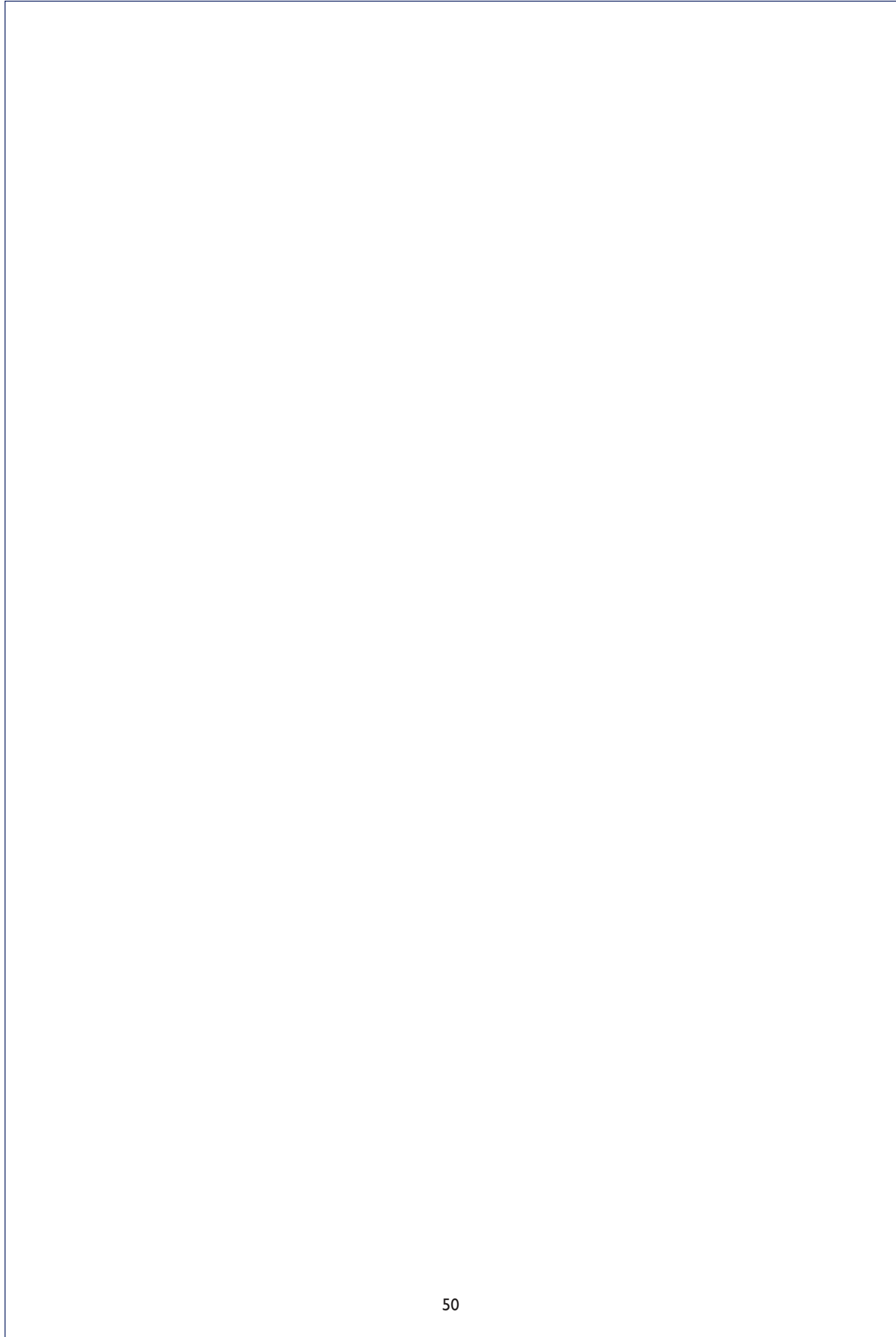
5.1. Analytical Services

All analytical services can be realized as screening / ranking, exploratory or development assays. GLP and non-GLP services are available.

- analytical method development and validation for NCEs
- performance and analysis of phase I and phase II enzyme marker reactions
- analysis of *in vitro* samples
- analysis of *in vivo* samples from human and animal studies (plasma, urine, etc.)

5.2. Instrumentation

- HPLC-DAD / FLD
- LC-MS / MS
- radiodetection
- UV / VIS spectrophotometer (cuvette / MTP)
- fluorescence reader (MTP)



Service Packages

6



6.1. Screening Package

6.2. Ranking Package

6.3. Metabolism Package

6.4. Drug-Drug Interaction Package

6.5. GenTox Package

6.6. Early ADME-Tox Test Strategy

6.6.1. Early Metabolism Testing

6.6.2. Early Toxicity Testing

6.6.3. Knowledge Based Decision Making

Please contact our experts to discuss your individual test strategy and service packages.

The service packages can be combined with in vivo tests and other partnered services.

6.1. Screening Package

- Cytotoxicity / Hepatotoxicity Screen
- Ames Screen
- Metabolic Stability Screen
- CYP Profiling Screen
- CYP Inhibition Screen

6.2. Ranking Package

- Cytotoxicity / Hepatotoxicity Screen
- Ranking Ames
- Metabolic Stability Screen
- Limited CYP Profiling
- Limited CYP Inhibition

6.3. Metabolism Package

- Metabolic Stability
- Metabolite Profile
- Enzyme Kinetics
- CYP Profiling

6.4. Drug-Drug Interaction Package

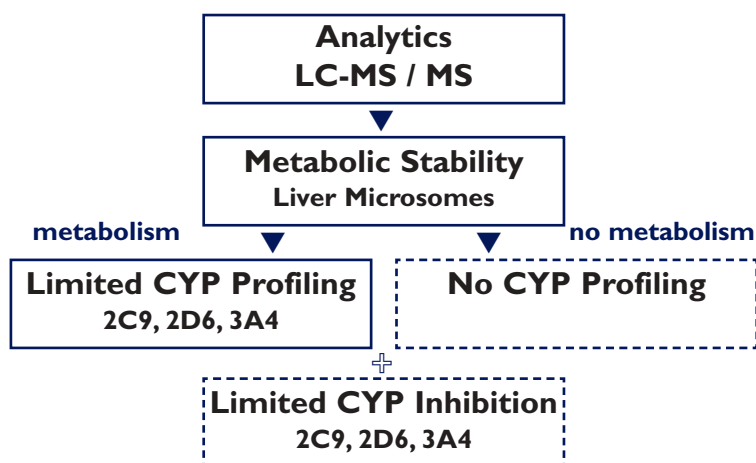
- CYP Inhibition Screen
- CYP Inhibition (IC₅₀)
- CYP Inhibition (K_i)
- CYP Induction

6.5. GenTox Package

- AMES Test
- Cytotoxicity
- Chromosome Aberration or Micronucleus Test
- Mouse Lymphoma Assay or HPRT Test

6.6. Early ADME-Tox Test Strategy

6.6.1. Early Metabolism Testing



Information

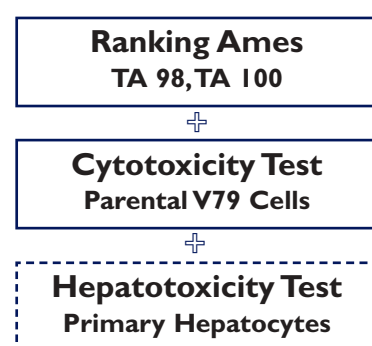
Selective Detection of NCE

Phase I Metabolism:
CYP Dependent Metabolism

Identification of Critical CYPs:

- 2C9, 2D6 + 3A4 Metabolize ca. 95% of all Drugs
- 2C9 + 2D6 are Polymorphic
- 3A4 is Frequently Involved in Drug-Drug Interactions

6.6.2. Early Toxicity Testing



Information

Bacterial Mutagenicity
Frameship and Point Mutations

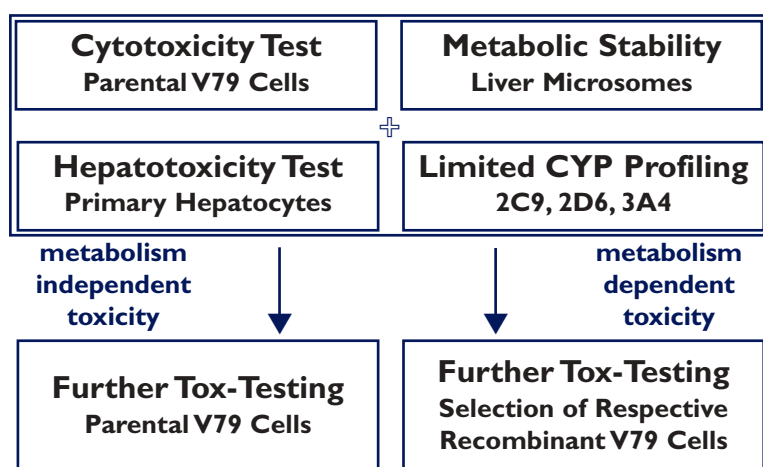
Metabolism Independent
Cytotoxicity

Metabolism Dependent &
Independent Cytotoxicity

Possible Add ons:

- Metabolite Profile (LC-MS) for Phase I + II Metabolism
- CYP Induction (1A2 + 3A4)

6.6.3. Knowledge Based Decision Making



Results of Early ADME-Tox Test Package

e.g., MN *in vitro*, HPRT, Cytotox, CA, Comet-Assay

OECD Recommendation: "A number of developments, including the construction of genetically engineered cell lines expressing specific activating enzymes, may provide the potential for endogenous activation. The choice of the cell lines used should be scientifically justified (e.g. by the relevance of the cytochrome P450 isoenzyme for the metabolism of the test substance)."

Partnered Services

7



7.1. Central Nervous System

- 7.1.1. CNS: *in vitro* Services
- 7.1.2. CNS: Neurophysiology
- 7.1.3. CNS: *in vivo* Services

7.2. Immunology / Inflammation

- 7.2.1. Immunology / Inflammation: *in vitro* Services
- 7.2.2. Immunology / Inflammation: *in vivo* Services

7.3. Cancer / Oncology

- 7.3.1. Cancer / Oncology: *in vitro* and *in vivo* Services

7.4. HIV-1 / AIDS

- 7.4.1. HIV-1 / AIDS: *in vitro* Services

7.5. Dermatology / Cosmetics

- 7.5.1. Dermatology / Cosmetics: *in vitro* Services

7.6. Oral Care

- 7.6.1. Oral Care: *in vitro* Services

7.7. Partnered Services / Contacts / Network

7.8. Consulting

7.1. Central Nervous System

The CNS services are offered in co-operation with VivaCell.

7.1.1. CNS: *in vitro* Services

Assays for the investigation of inflammation, pain, fever, depression and neurodegenerative disorders like Alzheimer's disease, multiple sclerosis, etc. are offered.

Models

- primary microglia / astrocytes / neurons (rat, murine)
- U373 MG (human astrocytoma cell line), human neuronal cell lines
- brain slice cultures
- 293T and CHO cells with heterologue expression of the Vanilloid Receptor type 1 (VR-1)

Parameters

- Prostaglandins (e.g. PGE2), cyclooxygenase expression and activity (COX-1, COX-2)
- iNOS/ NO release
- cytokines (TNF- α , IL-6, etc)
- growth factors (NGF, etc.)
- neuropeptides (substance P, etc.)
- transcription factors (NF- κ B/I κ B, AP-1, STATs, etc.)
- kinases (Erks, Protein kinase C, Jun, p38 MAP), etc.
- neurotransmitters (e.g. serotonin, noradrenalin, dopamin re-uptake)
- proteasome activity
- calcium mobilization, cytotoxicity and apoptosis, determination of ROS and mitochondria transmembrane potential
- agonist and antagonist of the VRI for non-opiaceous analgesic pharmacophores
- receptor assays (screening, membrane preparations, cells, dose-response)

7.1.2. CNS: Neurophysiology

Models

- primary slice cultures of guinea pig

Parameters

- measurements of transmembraneous calcium and potassium currents in CI pyramidal cells, interneurons and neurocortical pyramidal cells
- associative and non associative LTP and LTD in the hippocampus
- firing pattern in hippocampal and neurocortical cells
- pharmacological screening via associative LTP
- models of epileptic activity

7.1.3. CNS: <i>in vivo</i> Services	
Highly specialised and reliable preclinical <i>in vivo</i> disease models for drug development are offered.	
Stroke	■ transient or permanent occlusion of the middle cerebral artery in rat, mouse, and transgenic rodents, focal ischemia in gerbils
Cardiac Arrest	■ occlusion of both carotid and vertebral arteries in rat and gerbil
Head Trauma	■ controlled cortical impact injury in rat and mouse
Spinal Cord Trauma	■ with a spinal cord impactor in rat
Parkinson's Disease	■ intracerebral injection of 6-OH dopamine in rat and mouse
Epilepsy	■ systemic / hippocampal kainic acid injection in rat and mouse
Huntington's Disease	■ intrastriatal injection of quinolinate in rat and mouse
Migraine	■ spreading depression induced by KCl in rat and mouse
Brain Edema	■ cold-injury-induced in rat and mouse
Neuromuscular and Neurological Models	
	■ multiple sclerosis (EAE), transgenic models for ALS ■ inherited murine models of motoneuron diseases
Peripher Neuropathy	■ sciatic nerve crush ■ diabetic rats (streptozotocin induction) ■ acrylamide and cisplatin-induced neuropathies
Pain	■ chronic or acute pain
Anxiety	■ free exploratory test ■ white / dark box ■ elevated plus maze ■ open-field
Depression	■ chronic mild stress ■ forced swim test
Schizophrenia	■ prepulse inhibition memory & amnesia ■ conditioned avoidance responses (passive avoidance) ■ maze learning tasks (radial maze, T-maze) ■ object recognition task ■ spontaneous and delayed alternation tasks (T-maze) ■ operant bar-press task
Anhedonia, Hedonia	■ place preference conditioning

7.2. Immunology / Inflammation	
The immunology / inflammation services are offered in co-operation with VivaCell.	
A wide portfolio of protocols to evaluate the natural and specific immune response in isolated human and murine lymphoid cells and also in transformed cell lines is offered. In addition, animal models mimicking human pathologies are also available. These <i>in vivo</i> and <i>in vitro</i> models are suitable for preclinical validation of anti-inflammatory and immunomodulatory compounds.	
7.2.1. Immunology / Inflammation: <i>in vitro</i> Services	
Models	<ul style="list-style-type: none"> ■ primary human monocytes (rheumatic diseases, wound healing, etc.) ■ primary T-/B-lymphocytes, neutrophils, Natural Killer cells ■ primary fibroblasts, keratocytes, melanocytes ■ primary endothelial cells (heart) and HUVEC ■ murine thymocytes and primed T cells from spleen and lymphatic nodes of antigen-stimulated mice ■ enrichment of dendritic cells ■ chondrocytes ■ transformed T cell lines, B cell lines and macrophage cell lines
Parameters	<ul style="list-style-type: none"> ■ Prostaglandins (PGE2), cyclooxygenases (COX-1, COX-2), 5-lipoxygenase and determination of leukotriens by ELISA, activity of cPLA2 ■ cytokines by ELISA or RT-PCR (monocytes: TNF-α, IL-6, IL-1, IL-8, etc.; T-cells: IL-2, IL-4, γ-IFN) ■ iNOS/NO ■ growth factors ■ proliferation/activation of T-/B-lymphocytes ■ phagocytic activity ■ coagulation assays (aPTT, PT, TZ, Fxa) ■ matrix metalloproteases (MMPs: MMPI, MMP3, MMP9 etc.) ■ transcription factors (NF-κB, NF-AT, AP-1, OCT-1 and STATs) ■ signaling studies, MAPKs (ERK 1/2, p38, JKN/SAPK); PKCs; calcineurin dephosphorylation; cyclins activation, tyrosine kinases and the like ■ determination of phenotypes (CD determinations by FACS analysis): single and double staining ■ proliferation and cell cycle analyses by incorporation of 3[H]TdR/BrdU and/or FACS analysis: (a) mitogens (PHA, ConA, LPS); (b) TCR specific, CD3 or CD3/CD28 or with the superantigen SEB; (c) cell cycle analyses: progression to the S-phase and G2/M of the cell cycle

- antigen specific proliferation in murine primed T cells, Th1 and Th2 profile by cytokine release
- allogenic T cell proliferation (mixed lymphocyte cultures)
- induction of antigen-specific antibody production *in vitro*
- natural Killer activity and CTL activity
- determination of cell death in primary cells by determining either necrosis or apoptosis (type I and II)
- signalling studies in primary T/B cells and in lymphoid tumor cells: calcium mobilisation, intracellular pH changes, and the like
- transfections in primary T cells and in lymphoid cell lines: functional studies of transcriptional regulation using a big panel of cellular and synthetic promoters cloned in front of the luciferase gene (NF- κ B-Luc, AP-1-Luc, NF-AT-Luc, IL-2, TNF, IL-6, ICAM-1, CD69, cyclins, p21, Bax, PECAM, etc.)
- stably transfected cell lines with the luciferase gene driven by several inducible promoters regulated by PMA, IL-1 and TNF α -transcription factors (NF- κ B, LTR-HIV)

7.2.2. Immunology / Inflammation: *in vivo* Services

Models

- Inflammatory Bowel Disease (IBD):
A model for intestinal inflammation in Swiss mice comparable to Morbus Crohn and colon ulcerative diseases. The parameters to measure are morphological and histopathological.
- Septic Shock:
Induced by LPS in Balb/c mice and the parameters to measure are survival and measurement of IL-1, TNF α , and IL-6 in serum. Transcription factors are also measured in isolated spleen cells of treated mice.
- Collagen Induced Arthritis:
Morphological and histopathological criteria and phenotypical analysis of inflammatory infiltration in affected joints.
- Murine Models for Immunoglobulin Measurement:
In vivo isotype switching, IgG and IgE
- Skin Allograft Rejection
- Delayed-Type Hypersensitive Reaction:
Murine model for human allergic contact dermatitis.

7.3. Cancer / Oncology

The cancer / oncology services are offered in co-operation with VivaCell.

7.3.1. Cancer / Oncology: *in vitro* and *in vivo* Services

Models

- *In vitro* testing methods include proliferation inhibition assays using human tumor cell lines, as well as clonogenic assays with human tumor xenografts, human tumor cell lines, and hematopoietic stem cells. More than 350 solid tumor models have been established in serial passage as well as 20 permanent tumor cell lines (co-operation). In addition, a panel of 60 cell lines, commonly used in cancer research such as A549, MCF-7, Caco-2, HT29 or PC3 are also available. Cancer cell lines over-expressing the MDR1 gene are also available for drug research.
- *In vivo* assays include subcutaneous models, orthotopic models with human xenografts, murine tumour models (co-operation) and models of topic co-carcinogenesis inducing papilloma-like skin tumours in athymic mice.

Parameters

- proliferation, XTT assays, determination of DNA content by FACS analyses
- cytokines (e.g. IL-6), growth factors (e.g. EGF), etc.
- angiogenesis (e.g. VEGF, angiopoietin I and II, nephrin, i.e.): *in vitro* models of angiogenesis using HUVECs and Matrigel Basement Membrane Matrix; *in vivo* models of angiogenesis by corneal neovascularization assays
- cell cycle analyses, cytotoxicity by measuring PI staining, determination of DNA fragmentation (apoptosis) by TUNEL
- transmembrane potential of the mitochondria, calcium and pH mobilisation
- reactive oxygen species (ROS)
- Fas clustering and signalling in type I and II cells for apoptosis
- caspases (3, 8, 6 and 9) activity by spectrofluorometry and western blots
- Bcl-2, Bcl-X, Bax, Bid and cytoplasmic cytochrome C by western blots
- apoptosis and cell cycle analyses in MDR cell lines
- phosphatidyl serine expression at the cell surface by Annexin V binding
- integrity of tubulin and actin by fluorescence microscopy
- *in vitro* assays for tubulin polymerization
- cyclins activity by IP and western blots
- activation of transcription factors

- signalling studies MAPKs, IKK
- determination of telomerase activity in HeLa cells by telomerase PCR ELISA
- determination of estrogenic or anti-estrogenic activity of phytoextracts using ER expression vectors (α γ β subunits) and a specific ER responsiveness promoter driven the luciferase gene
- customized “knock out” cell lines for specific genes
- DNA microarrays for cancer-related genes in tumoral cell lines

Other specific cellular and molecular techniques are available upon request.

7.4. HIV-1 / AIDS

The HIV-1 / AIDS services are offered in co-operation with VivaCell.

7.4.1. HIV-1 / AIDS: *in vitro* Services

In vitro testing methods include cell lines specific for HIV-1 studies.

Models

- Jurkat cells stably transfected with the luciferase gene directed by the LTR-HIV promoter
- MT-2 cells for infection with the HIV-1 virus
- Tat-transfected cells, *in vitro* infection of primary T cells
- determination of IC-50 for anti-HIV drugs using an artificial proviral clone

Parameters

- inhibition of cell death by HIV-1 in MT-2 cells (MTT assays)
- determination of p24 antigen in culture supernatants of primary T cells infected with HIV-1
- determination of the transcriptional activity of the LTR-HIV promoter by Tat and by TNF α
- determinations of anti-HIV compounds targeting Tat/TAR interaction and RNA elongation
- determination of viral package in 293T cells

7.5. Dermatology / Cosmetics

The dermatology / cosmetics services are offered in co-operation with VivaCell.

Highly specialised and reliable skin models for testing cosmetics and pharmaceutical compounds (phytopharma) are offered. Modern and standardised *in vitro* protocols according to ethical guidelines provide the advantage over *in vivo* traditional models. Selected *in vivo* models are also available.

7.5.1. Dermatology / Cosmetics: *in vitro* Services

Models

- animal skin organ cultures
- bovine and rabbit isolated cornea
- human normal keratinocyte monolayer cultures
- human normal fibroblast monolayer cultures
- red blood cells
- reconstituted human epidermis
- cell lines: keratinocytes (HaCaT, NCTC2544, ...), fibroblasts (MRC5, 3T3, ...), Madin Darby Canine Kidney (MDCK), etc.

Toxicity

Parameters

- cytotoxicity:
MTT
Neutral Red
- membrane damage:
red blood cell lysis
protein denaturation

Trans-Epithelial Permeability Effect

Parameters

- changes in permeability is measured as the leakage of fluorescein through an epithelial barrier:
cornea
skin
confluent cell monolayer

Phototoxicity or Protective Effects

Parameters

- changes in cell cultures or skin after a UV or visible light exposition:
cytotoxicity and membrane damage
oxygen radical production
glutathion S-transferase activity of cells
the complement photoactivation assay

Skin Firmness

Parameters

- measurement of adhesion molecules in cell cultures:
keratinocytes
fibroblasts

Extracellular Matrix Adhesion Assay

Parameters

- fluoresceinated cells are cultured on chambers coated with artificial extracellular matrix; fluorescence retained in such chambers after a short incubation period gives a measure of the capacity of cells to adhere in the presence of a test compound

Human Skin Fibroblast / Collagen Lattice Cytotoxicity Test

Parameters

- skin fibroblasts are incorporated into 3-d collagen lattices containing the test compounds; an inhibition of lattice contraction gives an indication of the deleterious effect of the compound under test

Healing and Migration Assay

Parameters

- the capacity to regenerate a "wound" in a cell monolayer culture or the capacity of cell to migrate across a porous membrane is measured

7.6. Oral Care

The oral care services are offered in co-operation with VivaCell.

7.6.1. Oral Care: *in vitro* Services

Models

- human primary monocytes
- human normal fibroblast monolayer cultures (comparable to gingival fibroblasts)
- primary oral gingival epithelial cells (treated with pathogens) cell lines: fibroblasts (MRC5, 3T3), etc.
- VRI transfected cells: activation of these cells through this nociceptive receptor could mimic the initial steps in neurogenic inflammation and it is suitable to test new analgesic compounds topically applied in oral care

Parameters

- inflammatory parameters: PGE2, LTB4, cytokines, etc.
- cytotoxicity: MTT, LDH, Neutral Red, etc.
- oxygen radical production
- cell proliferation
- angiogenesis
- healing and migration
- calcium mobilisation and cell death through agonists / antagonist of the VRI in a heterologous system

7.7. Partnered Services / Contacts / Network

- Physicochemical Properties
- Chemical Synthesis of Chiral and Non-Chiral Compounds
- NMR-Spectroscopy
- PAH-Analytics
- Phytopharmaka / Plant Extracts
- Nutrition
- Safety Pharmacology
- Inhalative Toxicology
- Phenotyping / Genotyping
- Clinical Studies

Please inquire for further partnered services.

7.8. Consulting

Both scientific and legal/regulatory expertises are necessary to establish streamlined test and regulatory submission strategies for your compounds.

GenPharmTox serves you as an experienced and accessible guide through the maze of scientific and governmental regulations.

Whether your company just needs advice, or more in-depth assistance, GenPharmTox is ready to provide custom resources to get the job done. We provide reliable and thorough assistance in navigating through the complex rules and regulations during drug discovery and drug development phases and a professional service for registration of your chemicals and drugs.



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