WuXi AppTec

_in Vitro_ Toxicity Services

Identify potential liability issues in early stages of drug discovery

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**Introduction to In Vitro Toxicity**

Drug induced toxicity is one of the leading causes of drug candidate failure in preclinical and clinical testing stage, and also the major reason for the withdrawal of approved drugs from the market. Initial toxicity testing is required during the nonclinical phase of development and relies primarily on animal studies. While these animal models have provided useful information on the safety of chemicals, they are relatively expensive, low-throughput, and sometimes inconsistently predictive of human biology and pathophysiology because of the species difference. With increasing numbers of new chemical entities for environmental and pharmaceutical uses, it is necessary to find a rapid and efficient method to screen chemicals for their potential toxicities. Since most drug-induced toxicity is due to toxic effects at the cellular level, alternative in vitro models are increasingly being used to estimate in vivo responses, to reduce and/or replace in vivo animal testing, and to increase the throughput. This idea is supported by the 2007 NRC report, "Toxicity Testing in the 21st Century (TT21C): A Vision and a Strategy." This report predicts substantial advances in toxicity testing in the near future which are much more specific and predictive of human toxicity. In vitro toxicity testing studies are faster, simpler and more scalable so they can be used in the early drug discovery stage to predict potential risk. This would not only be economical but ethical as it could markedly reduce the number of animal usage.

To address the need for early stage in vitro toxicity testing, WuXi AppTec Biology offers a panel of toxicity assays at the cellular level by utilizing cutting-edge technologies, such as conventional and automated patch clamp and high content screening (HCS). Applying these assays to your lead ID and optimization strategy can help provide a more thorough analysis of the severity and specificity of toxicity. This information can then be used to guide candidate compounds through the planning and execution of downstream in vivo tests.

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Cardiotoxicity

Unintended drug-induced arrhythmia, in particular Torsade de Pointe arrhythmia (TdP), have been responsible for approximately 21.4% of drug withdrawals from markets between 1990 and 2012. For the past decade, cardiac safety screening studies have been conducted according to ICH S7B and ICH E14 guidelines. The ICH S7B guideline includes an in vitro IkR (hERG) assay and an in vivo ECG assay to identify the potential for delayed repolarization (QT interval prolongation). Although hERG is the most important channel related to the risk of TdP, hERG screening alone cannot reliably detect potential cardiac adverse side effects. Furthermore, this over-simplified and highly sensitive approach can result in unwarranted attrition of novel drug candidates owing to false-positive findings. Recently a new paradigm to examine cardiotoxicity called the Comprehensive in Vitro Proarrhythmia Assay (CiPA) was proposed to replace the current ICH S7B/E14 guidelines. The CiPA core assays include: 1. the assessment of drug candidate effects on multiple human ventricular ionic channels and in silico reconstruction of human heart ventricular action potential to predict the proarrhythmic risk; and 2. the confirmation study using human stem-cell derived cardiomyocytes²,³.

Comprehensive In Vitro Proarrhythmia Assay (CiPA)

<table>
<thead>
<tr>
<th>Ion Channel</th>
<th>Species</th>
<th>Assay platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>hERG (I_{Kr})</td>
<td>human</td>
<td>QPatch or Manual Patch Clamp</td>
</tr>
<tr>
<td>Cav1.2 (I_{Ca,L}-L-type)</td>
<td>human</td>
<td>Manual Patch Clamp</td>
</tr>
<tr>
<td>Nav1.5 (I_{Na}-peak current)</td>
<td>human</td>
<td>QPatch or Manual Patch Clamp</td>
</tr>
<tr>
<td>Nav1.5 (I_{Na}-late current)</td>
<td>human</td>
<td>QPatch or Manual Patch Clamp</td>
</tr>
<tr>
<td>Kv4.3/KChp2.2 (I_{K})</td>
<td>human</td>
<td>QPatch or Manual Patch Clamp</td>
</tr>
<tr>
<td>KCNQ1/mink (I_{K1})</td>
<td>human</td>
<td>Manual Patch Clamp</td>
</tr>
<tr>
<td>Kir2.1 (I_{Kr})</td>
<td>human</td>
<td>QPatch or Manual Patch Clamp</td>
</tr>
</tbody>
</table>

WuXi AppTec provides a combination of assay platforms that include manual and automated patch-clamp and microelectrode array (MEA) to access drug candidate effects on multiple cardiac ion channels (Table 1) as well as stem cell-derived ventricular cardiomyocytes as CiPA recommended.

![Diagrammatic representation of CiPA](image)

Table 1. Cardiac ion channel panel (CiPA recommended).

WuXi AppTec Biology obtains human induced pluripotent stem cell-derived ventricle cardiomyocytes (hiPSC-vCMs) from a third party vendor. The purity of the ventricular cardiomyocytes is over 90%, which is the highest number seen in the literature. The action potential, main ion channel properties and development process of the hiPSC-vCMs have all been fully validated using the manual patch clamp system at WuXi AppTec. WuXi AppTec Biology now offers CiPA confirmatory electrophysiological test³ with MEA (Maestro Pro, Axion) to analyze the effects of compounds on hiPSC-vCMs.

In addition, to satisfy the mechanistic study or high throughput study needs, WuXi AppTec Biology also provides traditional radioligand binding and fluorescence signal detection assays. The service for some other cardiac targets such as Kv1.5 (Ikur) and Cav3.2 (ICa,T) is also available.
Cytotoxicity testing is mandated by the FDA and CFDA for IND/CTA submission and is typically performed during the nonclinical phase of discovery. In recent decades, it is well accepted that in vitro cytotoxicity testing methods should be considered before animals are dosed to examine acute oral systemic toxicity. Identifying potential cytotoxicity early in the process can dramatically save both time and capital by eliminating likely toxic compounds prior to pivotal animal studies. Additionally cell-based assays are easy to scale for medium or high throughput screening and guiding go/no-go decisions.

- **Cell Viability: ATP production measured in active cells**

(A) In a 384-well HepG2 cell viability assay, 24 hours of Nefazodone treatment inhibited cell viability in a concentration dependent manner (n=5).

(B) After 72 hours treatment in 384-well plates, Staurosporine showed concentration dependent inhibition on the viability of HepG2 cells (n=5).

The CellTiter-Glo® Luminescent Cell Viability Assay (Promega): This is a homogeneous method to determine the number of viable cells based on quantitation of the ATP present, which is a marker for the presence of metabolically active cells. WuXi AppTec Biology has fully validated this assay with a wide range of cell types, in both 96- and 384-well format, using EnVision (Perkin Elmer) as the luminescent signal reader.

- **Apoptosis: Caspase assay**

Activation of the caspase cascade is an integral event in the apoptotic pathway. WuXi AppTec Biology uses Caspase-Glo® 3/7 Assay kit (Promega) to measure caspase-3 and caspase-7 activities. The assay was fully validated on HepG2 Cells with EnVision as the Luminescence signal reader. Available in both 96- and 384-well format.

Staurosporine increased caspase activity. In a 96-well plate assay staurosporine concentration-dependently increased caspase activities in the HepG2 cells. EC_{50} was 0.07 μM and 0.23 μM for 6-hour and 18-hour staurosporine treatment, respectively.

- **CellTox™ Green Cytotoxicity Assay (Promega)**

The CellTox™ Green Cytotoxicity Assay measures changes in membrane integrity that occur as a result of cell death. The assay system uses a proprietary asymmetric cyanine dye that is excluded from viable cells but preferentially stains dead cell DNA. Viable cells produce no appreciable increases in fluorescence. Therefore, the fluorescent signal produced by the dye binding to the dead-cell DNA is proportional to cytotoxicity. We now offer this assay in both 96- and 384-well format on various cell types, using Envision as the signal detector. This assay can be combined with CellTiter-Fluor™ Cell Viability Assay or CellTiter-Glo® Luminescent Cell Viability Assay (Promega).

CellTox™ Green Dye binds DNA of cells with impaired membrane integrity.
LN-18 cells were exposed to staurosporine with indicated concentrations in a 384-well assay plate for 24 hours. Fluorescence associated with cytotoxicity was measured after CellTox™ Green Reagent was applied, then the CellTiter-Fluor Reagent was applied and fluorescence signal was measured. These measurements produced similar EC50 values.

- **ApoTox-Glo™ Triplex Assay (Promega)**
  
  The ApoTox-Glo™ Triplex Assay combines three Promega assay chemistries to assess viability, cytotoxicity and caspase activation events within a single assay well. The first part of the assay simultaneously measures two protease activities; one is a marker of cell viability, and the other is a marker of cytotoxicity. The second part of the assay uses the Caspase-Glo® Assay Technology to detect caspase activities. WuXi AppTec has validated this assay with various cell types, using Envision as the signal reader.

- **Mitochondrial Toxicity**

  Mitochondria play a pivotal role in cellular energy (ATP) production and maintaining homeostasis. Mitochondrial dysfunction is increasingly implicated as a major contributor to drug-induced toxicity, leading to the discontinuation of prominent drugs, including troglitazone, cerivastatin and nefazodone. In addition to post-market drug withdrawals, mitochondrial liabilities have also been associated with many drugs carrying a black box label for hepatic and cardiac toxicity.

  WuXi AppTec offers a set of in vitro assays to access mitochondrial toxicity from different endpoint.

  - **Mitochondrial Membrane Potential Assay**
    
    Mitochondrial membrane potential (MMP) is tightly interlinked to many mitochondrial processes so it is a key indicator of mitochondrial function and cell health. The dissipation of MMP is considered an early indicator of apoptosis.

    WuXi Biology offers a plate based HCS assay to detect the MMP, using the Acumen Cellista (TTP Labtech) with MITO-ID® Membrane Potential Cytotoxicity Kit (ENZO Life Sciences). The assay is available in both 96- or 384-well format, and in a wide range of cells.

    The MITO-ID® Membrane Potential Cytotoxicity Kit utilizes a cationic dual-emission dye that exists as green fluorescent monomers in the cytosol, and accumulates as orange fluorescent aggregates in the mitochondria. Cells exhibit a shift from orange to green fluorescence as mitochondrial function becomes increasingly compromised.
Mitochondrial Reactive Oxygen Species (ROS) Assay: ROS-Glo™ H₂O₂ Assay

Mitochondrial dysfunction usually causes increased free radical production. The predominant source of free radical generation is the mitochondrial respiratory chain, and inhibition of this process is often connected to increased levels of reactive oxygen species (ROS). In the different ROS generated in cell culture, H₂O₂ is convenient to assay because of the long half-life in cultured cells. A change in H₂O₂ can reflect a general change in the ROS level.

WuXi Biology has validated a plate based assay using ROS-Glo™ H₂O₂ assay kit (Promega) to detect H₂O₂ levels. The assay is available in both 96- or 384-well format, and can also be combined with other cytotoxicity measurements.

Oxygen Consumption Assay: MitoXpress® Xtra OCR Assay (HS method) (Luxcel)

The MitoXpress® Xtra assay directly measures immediate and acute drug effects on mitochondrial oxidative phosphorylation (Oxygen Consumption Rate, OCR) and is the most effective high throughput screen for Mitochondrial Toxicity using whole cells. Oxygen consumption is the most important parameter for the direct and specific assessment of the function of the electron transport chain, the cornerstone of oxidative phosphorylation and cellular metabolism. In the MitoXpress® Xtra OCR assay, the MitoXpress® reagent is quenched by oxygen, whereby oxygen depletion caused by mitochondrial activity causes an increase in probe signal, with rates of oxygen consumption calculated from the changes in fluorescence signal over time. Available in a 384- or 96-well high throughput format, this assay is compatible with a very wide range of primary, iPS or cell line models and both 2D and 3D culture systems.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀(μM)</th>
<th>Literature (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotenone</td>
<td>0.029±0.007</td>
<td>0.020±0.007</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>13.90±0.88</td>
<td>12.65±2.43</td>
</tr>
</tbody>
</table>

Results from a typical 96-well MitoXpress® Xtra (HS method) OCR concentration-response assay for two mitochondrial inhibitors, Rotenone and Nefazodone. The HL60 cells were used in the assay.
• **MitoXpress® Cellular Energy Flux OCR/ECAR Assay (Luxcel)**

A deeper, investigative analysis to understand the mechanism of mitochondrial toxicity and its relationship to cellular ATP production is made possible through the addition of Luxcel Biosciences pH Xtra – Glycolysis Assay; detecting in real-time the combined effects on OCR and extracellular acidification rate (ECAR). This assay is available in a 384- or 96-well high throughput format.

![Results from typical 384-well MitoXpress® Cellular Energy Flux OCR/ECAR assay with HL60 cells, illustrating the difference between a classic mitochondrial inhibitor, Rotenone, which decreased OCR / increased ECAR (A); and a non-specific cytotoxic drug, Tamoxifen, which decreased both OCR and ECAR (B).](image)

• **Glucose/Galactose Assay**

Replacing glucose with galactose in the cell media increases the reliance of the cells on mitochondrial oxidative phosphorylation, thereby increasing susceptibility to the implications of mitochondrial insult. By comparing the differential toxic effects on glucose and galactose grown cells it will differentiate mitochondrial toxicity from non-specific cytotoxicity.

We use HepG2 cells cultured in either glucose (25 mM) or galactose (10 mM), with cytotoxicity assessed using CellTitre-Glo™ (Promega). A mitochondrial toxicant is indicated by a greater than three-fold change in IC₅₀ value observed in the glucose media compared to the galactose media.

![Illustration of the 24-hour treatment data for the mitochondrial uncoupler, FCCP (A), and inhibitor, nefazodone (B). A > 3-fold and 3.7-fold increase in IC₅₀ value is observed for FCCP and nefazodone, respectively, in glucose media compared with galactose media.](image)

• **MitoBiogenesis Assay**

Determination of the mitochondrial biogenesis level relative to the cellular protein synthesis provides important information on potential mitochondrial toxicity. This is particularly important for antiviral and antibiotic new drug development because the similarity between mitochondrial biogenesis and bacterial/viral replication. Many such drugs can cause serious mitochondrial toxicity.

Our mitobiogenesis assay uses Odyssey (LI-COR) with MitoBiogenesis™ In-Cell ELISA Kit (IR) (Abcam). The assay has been validated on HepG2 cells and are available in both 96- and 384-well format.

![This assay simultaneously measures the levels of two mitochondrial proteins, Mitochondrial DNA encoded COXI and nuclear DNA encoded SDH-A. The specific inhibition of Mitochondrial DNA encoded protein synthesis by chloramphenicol is thus easily observed.](image)
Inhibition of mitochondrial biogenesis by chloramphenicol. (A) HepG2 were seeded at 1200 cells/well in 384 well plate. Chloramphenicol inhibits COX-I protein synthesis relative to SDH-A protein synthesis. (B) The overall mitochondrial biogenesis inhibition was calculated from the ratio of measured COX-I/SDH-A protein levels.

**Lipotoxicity**

Phospholipidosis is a lysosomal storage disorder and characterized by the accumulation of excess phospholipid complexes within the internal lysosomal membranes. Cationic amphiphilic drugs (CADs), such as antibiotics, antidepressants, antihistamines and other prescription drugs, have been identified as inducers of phospholipidosis. The US FDA has acknowledged that drug-induced phospholipidosis is an adverse drug reaction.

Steatosis is the situation of cytoplasmic accumulation of neutral lipids. Some drug can interfere with hepatic lipid processing, leading to accumulation of triglycerides within the liver cells. This condition may lead to harmful liver inflammation, or steatohepatitis.

Both drug induced phospholipidosis and steatosis are often reversible conditions without remarkable consequences; however, after prolonged exposure to a particular drug, they can lead to long-term toxic effects. Therefore drug induced cellular lipotoxicity leading to phospholipidosis and/or steatosis should be evaluated during the early drug discovery stage to minimize potential risk.

WuXi AppTec now offer in vitro HCS assays on HepG2 cells using the HCS LipidTOX™ Stains (Thermo Fisher Scientific), with the CQ1 (confocal quantitative image cytometer, Yokogawa Electric Corporation), or Acumen Cellista (TTP Labtech) as the image reader. Phospholipidosis is detected with the LipidTOX™ Red phospholipid stain, while steatosis is detected with the LipidTOX™ Green neutral lipid stain.

- **Phospholipidosis assay**

HepG2 cells were exposed to test articles and LipidTox reagent in a 96-well plate for 48 hours. Hoechst 33342 nuclear staining was used as control. Fluorescence images were captured with CQ1. LipidTox red phospholipidosis and Hoechst 33342 emit red and blue fluorescence in the same field, respectively.
In Vitro Toxicity Services

Results for red fluorescence were normalized to those of blue fluorescence (Hoechst). Propranolol concentration-dependently increased phospholipid staining.

● Steatosis assay

HepG2 cells were exposed to the test articles in 96-well plate for 48 hours. Fixed cells were stained with LipidTOX™ Green neutral lipid stain and the Hoechst 33342. Fluorescence images were captured with CQ1. The Green Neutral Lipid Stain and Hoechst 33342 emit green and blue fluorescence in the same field, respectively.

Results for green fluorescence (LipidTox Green Neutral Lipid Stain) were normalized to those of blue fluorescence (Hoechst). Both Cyclosporin A and Loratadine concentration-dependently increased the Green Neutral Lipid Stain, suggesting the accumulation of steatosis.

Hepatotoxicity

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Drug-induced liver injury (DILI) is caused by many hundreds of widely prescribed drugs and is a leading cause of drug development and registration failure, withdrawal of approved drug, and cautionary labeling which restricts drug usage. The assessment of compound-induced hepatotoxicity has traditionally relied on in vivo testing; however the studies are limited to a small amount of late stage compounds. In addition there are species-specific differences. Since most severe DILI is due to hepatocellular injury, in vitro hepatotoxicity testing would provide valuable information to predict potential risk.

WuXi Biology offers multiplexed in vitro assays to assess potential hepatotoxicity at cellular level. The assays use HepG2 (human liver hepatocellular carcinoma) cells, or human primary hepatocytes. By using these cells as in vitro models, all assays listed in the General Cytotoxicity, Mitochondrial Toxicity, and Lipotoxicity sections in this brochure can be used to evaluate potential hepatotoxicity. The combination of these assays will provide valuable information from different endpoint to analyze the mechanism and severity of hepatotoxicity.
Recommended HepG2 hepatotoxicity first tier assays:

- Cell Viability Assay: a very sensitive marker to detect general toxicity;
- Mitochondrial Membrane Potential Assay: an indicator of poor respiratory capacity and cell health;
- Mitochondrial Reactive Oxygen Species (ROS) assay: ROS increase may result in significant cell structure damage and cause oxidative stress;
- Caspase assay: measuring Caspase-3/7 activities.

Assay Features and Advantages:

- Plate-based assay (96- or 384-well plate) read by plate reader or high content image reader;
- Medium to high throughput with high sensitivity;
- Low cost with a short turnaround time;
- Short term (24 h) and long term (10–14 d) toxicity assays available.

### HepG2 Cell Viability Assay

<table>
<thead>
<tr>
<th>Reference Compound</th>
<th>Staurosporine</th>
<th>Tamoxifen</th>
<th>Nefazodone</th>
<th>Rotenone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>72h</td>
<td>72h</td>
<td>24h</td>
<td>24h</td>
</tr>
<tr>
<td>IC50 (µM)</td>
<td>0.036 ± 0.007</td>
<td>10.55 ± 0.59</td>
<td>48.09 ± 7.11</td>
<td>4.49 ± 1.05</td>
</tr>
<tr>
<td>n</td>
<td>n=5</td>
<td>n=5</td>
<td>n=5</td>
<td>n=5</td>
</tr>
</tbody>
</table>

IC50 values of several reference compounds in our HepG2 cell viability assay.

### Nephrotoxicity

The kidney’s primary function is the filtration and excretion of soluble waste while retaining key biochemicals. Thus its design as a selective filter makes it particularly susceptible to toxic injury. Renal tubular cells, in particular proximal tubule cells, are especially vulnerable to drug toxicity due to their prominent role in the filtration process which exposes them to high levels of circulating toxins. Thus, our assays use human-derived cellular systems originating from the renal proximal tubule epithelial cell line (HK-2) to maximize the predictive power of nephrotoxicity. The HK-2 cells are derived from normal adult human kidneys and immortalized by transduction of the E6/E7 genes via HPV-6.

WuXi AppTec has developed several nephrotoxicity assays to enable high throughput screening providing an early assessment of nephrotoxic effects and the potential for kidney-specific cellular injury. In general, all assays listed in the General Cytotoxicity and Mitochondrial Toxicity sections above can be used to assess nephrotoxicity, including the Cell Viability Assay, Apoptosis assay, etc.

![Validation data on HK-2 cells using the CellTiter-Glo viability assay. The assay was run at 9 concentrations in triplicate and the dose response curve of paclitaxel is presented. Two reference compounds, paclitaxel and staurosporine were tested. The IC50 were 20 nM and 16 nM, respectively.](image)

### Genotoxicity

When DNA is exposed to particular chemicals, mutations and other damage can occur leading to cancer and/or teratogenic effects. The severity of these effects then necessitates examining whether new or existing chemicals intended for human use have any effect on DNA. This genotoxic potential is an integral part of the basic toxicological information package used in the decision-making and risk assessment process of drug development. Since no single test is capable of detecting all relevant genotoxic endpoints, a battery of tests for genotoxicity is recommended by regulatory agencies.
Mini Ames Assay

The mini Ames assay is modified from the standard Ames test that uses 6-well plates and 20% of the typical Ames assay medium. The purpose is to generate a rapid screening test that utilizes small quantities of the test compound but still gives results in agreement with those of the standard Ames assay. The mini Ames assay is widely used as an early compound screen during lead optimization or preclinical candidate selection.

WuXi offers the Ames Microplate format (MPF) Assay, which corresponds to the Ames Fluctuation Assay that is cited in the guidelines of OECD and FDA. The MPF assay uses a liquid format and 384-well microplates, which requires less test compound, is time and cost-effective, but still shows good correlation with the traditional plate incorporation assay. The MPF assay employs four Salmonella typhimurium TA98, TA100, TA1535 and TA1537, E. coli strain wp2 uvrA or wp2 uvrA [pKM101] for mutagenic potential investigation in the presence or absence of a metabolic activation system (e.g., Aroclor 1254-induced rat liver S9). It generates a rapid screening and increases the throughput for drug mutagenic potential assessment in the early stage.

Bacteria are preincubated with the test compound (6 concentrations, a positive and a negative control) in exposure medium supplied with sufficient histidine or tryptophan with or without S9 for 90 minutes. The culture is then diluted into pH indicator medium without histidine (for S. typhimurium) or tryptophan (for E. coli) and aliquoted into 48 wells of a 384-well plate. After 48 h incubation, cells undergoing the reversion to prototrophy will change the medium colour from purple to yellow, which can be detected visually or by microplate reader.

<table>
<thead>
<tr>
<th></th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>without S9</td>
<td>with S9</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Nitrofurane (2</td>
<td>46</td>
<td>11</td>
</tr>
<tr>
<td>(µg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Aminoanthracene (5µg/ml)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Relative mutagenic potential of reference compounds as detected by Salmonella typhimurium TA98 and TA100. The number in the table indicates the revertants/48 wells.

In vitro Micronucleus Assay

The in vitro micronucleus assay is another part of the recommended regulatory testing battery for genotoxicity as an alternative to the chromosomal aberration assay. This assay also involves the analysis of chromosomal irregularities, but unlike the chromosomal aberration assay which requires considerable training, micronucleus testing is much simpler, faster and scalable. The micronucleus test examines the presence of micronuclei in the cytoplasm during interphase. Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of mitosis.

Phototoxicity

Phototoxicity is defined as a toxic response from a substance applied to the body, which is either elicited or increased after subsequent exposure to light, or that is induced by skin irradiation after systemic administration of a substance. The regulatory guidelines for phototoxicity are covered by the ICH S10, and the OECD Guideline for the Testing of Chemicals 432: In Vitro 3T3 NRU Phototoxicity Test.

In vitro 3T3 NRU Phototoxicity Test

The in vitro 3T3 NRU phototoxicity test is used to identify the phototoxic potential of a test substance induced by the excited chemical after exposure to light. The test evaluates photo-cytotoxicity by the relative reduction in viability of cells exposed to the chemical in the presence versus absence of light. Now the test has been fully validated in WuXi Biology Group using the permanent mouse fibroblast cell line, Balb/c 3T3, following the protocol documented in the OECD (432) guideline. SOL 500 (Dr. Höhne AG) was used as the light source while SpectraMax2 (Molecular Devices) plate reader was used to collect data.
Results of two phototoxic compounds, chlorpromazine and amiodarone hydrochloride. For the result evaluation, the Photo-Irritation-Factor (PIF) was calculated using the following formula: \( \text{PIF} = \frac{\text{IC}_{50}(\text{-UV})}{\text{IC}_{50}(\text{+UV})} \). In this study the PIF values of the two compounds are consistent with the reference data in OECD (432) guideline. A test material is defined as having a potential phototoxic hazard if the PIF value is or greater than 5.

<table>
<thead>
<tr>
<th>Test article</th>
<th>IC(_{50})Dark (µg/mL)</th>
<th>IC(_{50})UVA (µg/mL)</th>
<th>PIF</th>
<th>Phototoxic</th>
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</thead>
<tbody>
<tr>
<td>Chlorpromazine</td>
<td>13.2±1.42</td>
<td>0.35±0.07</td>
<td>38.2</td>
<td>+</td>
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<tr>
<td>Amiodarone hydrochloride</td>
<td>17.3±0.61</td>
<td>2.79±0.59</td>
<td>6.2</td>
<td>+</td>
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References: