

## **In-vivo Pharmacology Assays - Safety**

### **1. General Safety Assays**

#### **1.1 Conditioned taste aversion**

In order to evaluate conditioned taste aversion causing property of NCE under evaluation, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=8), subject to test, water and saccharin intake will be analyzed.

#### **1.2 Visceral sickness**

In order to evaluate visceral sickness causing property of NCE under evaluation, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=10), subjected to test, kaolin consumption will be analyzed.

### **2. CNS Safety Assays**

#### **2.1 Hot Plate**

In order to evaluate analgesic property of NCE under evaluation, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=8), subject to test, the latency for hind paw or fore paw licking or jump or flutter behavior will be analyzed.

#### **2.2 Irwin Test**

In order to study the general behavior of NCE under evaluation in rats, three groups of animals (Control, Two dose levels of test compound; n=8), subject to test and parameters as per functional observation battery will be analyzed.

#### **2.3 Maximal Electroshock**

In order to evaluate anticonvulsant property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=8), subject to test and time for extensor and flexor phase will be analyzed.

#### **2.4 Open Field**

In order to evaluate the CNS stimulant and depressant property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=8), subject to test and distance traveled in border and center, number of rearing in the border and center, total distance traveled and total number of rearing will be analyzed

### **Suven Life Sciences Limited**

Serene chambers, Road - 5, Avenue-7, Banjara Hills, Hyderabad 500 034, INDIA

Tel: 91-40-23556039/38 Fax: 91-40-23541152

Contact: [nvsrk@suven.com](mailto:nvsrk@suven.com), [jasti@suven.com](mailto:jasti@suven.com)

## **2.5 PTZ induced convulsion**

In order to estimate anticonvulsant property of NCE under evaluation, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=8), subject to test and onset of clonic convulsion will be analyzed.

## **2.6 Rota rod**

In order to evaluate skeletal muscle relaxant property of NCE under evaluation, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=8), subject to test and latency to fall from the rotating rod will be analyzed.

## **3. GI Safety Assays**

### **3.1 Gastric emptying**

In order to evaluate effect of NCE under evaluation, on gastric emptying, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10), subject test and % gastric emptying will be analyzed.

### **3.2 GI irritation**

In order to evaluate gastric irritation causing property of NCE under evaluation, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=8), subject test and the number of lesions and their size (in mm) will be analyzed.

### **3.3 GI secretion**

In order to evaluate effect of NCE under evaluation, on gastric secretion, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=8), subject to test and acidity, pH and volume of gastric contents will be analyzed.

### **3.4 GI transit**

In order to evaluate effect of NCE under evaluation, on gastric transit, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=8), subject to test and distance travelled by charcoal will be analyzed.

## **4. Respiratory Safety Assays**

### **4.1 Head out plethysmograph**

In order to evaluate effect of NCE under evaluation, on respiratory parameters, two groups of animals (Two dose levels of test compound; n=8), subject to head out plethysmograph test and respiratory rate (RR), tidal volume (TV), minute volume (MV), mid expiratory tidal volume (EF50), time of inspiration (Ti), time of expiration (Te), peak inspiratory flow (PIF), peak expiratory flow (PEF) will be analyzed.

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## **4.2 Whole body plethysmograph**

In order to evaluate effect of NCE under evaluation, on respiratory parameters, two groups of animals (Two dose levels of test compound; n=8), subject to whole body plethysmograph test and respiratory rate (RR), tidal volume (TV), minute volume (MV), mid expiratory tidal volume (EF50), penh, time of inspiration (Ti), time of expiration (Te), peak inspiratory flow (PIF), peak expiratory flow (PEF) will be analyzed.