TOXICITY ASSAY REPORT

Requesting Researcher: Paul Wrede

TYPE OF ASSAY

Toxicity assay with a single intravenous administration, testing three concentrations for the compound: 1 mg/ml, 2.5 mg/ml and 5 mg/ml. Number of compounds: 1.

SAMPLE PREPARATION

The compound was obtained in powder form and suspended in PBS at a working concentration of 5 mg/ml. From this suspension, two additional concentrations were prepared: 1 mg/ml and 2.5 mg/ml. All preparations were vortexed before each administration (Figure 1).

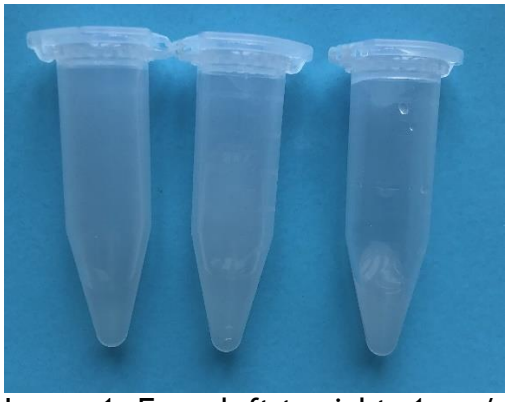

Image 1: From left to right: 1 mg/ml- 2.5 mg/ml- 5 mg/ml

DESIGN

Female Swiss mice, weighing approximately 20 g, were used. They were divided into four groups: G1: control, G2: 1 mg/ml, G3: 2.5 mg/ml, and G4: 5 mg/ml, with 3 animals per group (total = 12 animals).

All animals received 100 µl via intravenous administration.

CLINICAL MONITORING

Animals were observed for the appearance of clinical signs at the time of the administration and for at least 30 minutes thereafter.

Clinical follow-up lasted one week, with body weight recorded twice weekly. Signs of toxicity were recorded according to the following surveillance protocol:

Sign	**Clinical Assessment (score)**
Body Weight	
Weight equal or greater than baseline	0
5-10% weight loss	1
11-15% weight loss	2
16-20% weight loss	3
>20% weight loss	End point
Fur Condition (grooming failure)	
Good coat	0
Slightly unkempt fur	1
Mild piloerection	2
Marked piloerection	3
Clinical Signs: Respiratory Rate	
Normal	0
Mild dyspnea	1
Moderate dyspnea	2
Severe dyspnea	3

Behavior	
Normal behavior	0
Reduced behavior	1
Abnormal behavior	2
Self-mutilation	End point
Posture	
Normal posture	0
Antalgic posture	1
Presence of lameness	2
Immobile	3

Surviving animals at the endpoint (7 days post-administration) were euthanized, and samples were collected for hematological analysis (whole blood) and clinical biochemistry (serum).

RESULTS

Formulation 1 mg/ml

No signs of toxicity were observed in any of the administrations

Formulation 2.5 mg/ml

No signs of toxicity were observed in any of the administrations

Formulation 5 mg/ml

No signs of toxicity were observed in any of the administrations

After 24 hours, all the animals had formed nests. They displayed normal behavior (Image 2)

Image 2: From left to right: Control group- 1 mg/ml- 2.5 mg/ml- 5 mg/ml

TOXICITY INDICATORS (full results in attached file)

BODY WEIGHT EVOLUTION

None of the concentrations, under the tested conditions, caused a decrease in the animals' body weight.

CLINICAL BIOCHEMISTRY ANALYSIS

The most notable findings were observed at concentrations of 2.5 and 5 mg/ml, where a significant decrease in BUN (Blood Urea Nitrogen) levels was detected. Additionally, hyperglycemia was observed at the 5 mg/ml concentration. Since the remaining biochemical parameters were not altered, these findings are not considered toxicologically relevant.

HEMATOLOGICAL ANALYSIS

At all three concentrations, significant differences were observed in the main erythrocyte parameters: RBC (Red Blood Cells), HCT (Hematocrit), and HB (Hemoglobin). Due to the conditions of the study, it is difficult to establish a possible cause, and further in-depth studies would be required.

SUMMARY

Under the tested conditions (a single administration), the compound may cause mild effects on certain blood parameters. However, in the absence of clinical signs and changes in body weight, it can be concluded that this represents a non-significant acute toxicity, and in any case, these effects would be considered tolerable. Therefore, all three concentrations are considered to be below the Maximum Tolerated Dose (MTD).