

## Compound Safety Study

**Animal:** C57BL6 mice, BALB/c mice or another suitable animal.

**Experimental design:** Groups of 6 mice or an equally significant number (n=6) will be exposed to the test-compound at increasing doses, via the therapeutically meaningful route of administration to assess the safety profile of the test-compound. For example, in case of test-compounds administered intranasally, the animals are anesthetized prior to each administration using intraperitoneal ketamine/xylazine (50/5 mg/kg) – or another equally safe or improved anesthetic/sedative protocol - followed by intranasal instillation of 50 µl of three or more relevant doses of the test-compound in increasing concentrations. A mock group that receives only the vehicle must also be included in this study as a control group. Test-compound to be given once daily (qd) for 8 days. The weight of each individual animal has to be recorded every day beginning on the first day of the experiment. Daily observation for signs of toxicity and health deterioration, including overall survival has to be recorded meticulously. In the end, all the animals will be euthanized, and blood samples will be collected for hematology and clinical chemistry analysis. Organs and tissue including but not limited to nose, heart, liver, lungs, kidneys, spleen, gonads will be weighed and collected for histopathological examination.

Example of a study plan in the table below. Study can be adapted depending on the compound.

No./group	Group No.	Compound	Dose	Treatment Route & schedule	Observations/testing
3	1	Untreated			Daily body weight measurement and mortality observation.  At the end of study: Hematology and clinical chemistry analysis for blood samples Histopathological examination of organs and tissues including but not limited to nose, heart, liver, lungs, spleen, kidneys and gonads.
6	2	Vehicle		If intranasal administration (50ul in both nostrils) daily for 8 days	
6	3		Dose 1		
6	4		Dose 2		
6	5		Dose 3	Animals will be euthanized at day 16	