Final Report

Study Title	M58307: Single Dose Oral Toxicity Study in the Rat Approximation of the Minimum Lethal Dose Level
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SUMMARY

This study was conducted to estimate the approximate minimum lethal dose level of M58307 following single oral administration to the rat.

In the preliminary study, the test article was administered orally on Day 1 at dose levels of 71, 212 or 353 mg/kg to groups consisting of one male and one female fasted rat and at dose levels of 141, 159, 194, 229, 282 or 318 mg/kg to groups of two male and two female fasted rats. The surviving animals were killed on Day 8 and subject to a full necropsy. Deaths occurred in all groups dosed at levels higher than 141 mg/kg. There were no apparent effects on bodyweight gain arising from administration of any of the preliminary dose levels. Minor macroscopic changes noted during necropsy showed no trends indicative of a treatment related effect on any particular organ and were considered of limited toxicological significance.

The majority of clinical signs developed within the first hour following dosing for all of the preliminary groups and generally included the neuromuscular and breathing type changes. These typically included tonic convulsions, proneness, spasticity, twitching, ataxia, clonic convulsions, lethargy, tremors, paddling, abasia, arched or hunched posture and loss of movement due to limb rigidity and a stereotypic behavioural response which resulted in the animal biting the cage bars. The disruption to the animals breathing, probably resulting from convulsive episodes, was indicated by observation of tachypnoea, dyspnoea and/or hyperpnoea. Some of these signs were present, in varying degrees and at various group incidence, for animals dosed from 71 through to 353 mg/kg.

In addition, there were a number of other clinical signs that developed later in the observation period or were observed more sporadically or were considered to be of less clinical significance. Such signs included pilo-erection, salivation, staining of the snout or anogenital region, palpebral closure and exophthalmus. Later in the study, from Day 2 onwards some of the animals showed signs of increased activity, aggression and irritability. Clinical signs were relatively short-lived – animals dosed at 71 or 141 mg/kg had recovered by Day 2 and animals in the remaining groups had generally recovered by Day 3 to Day 5.

In the main study, fasted groups of five male and five female rats were given the test article as a single dose on Day 1 by oral gavage at dose levels of 265 or 141 mg/kg. The test article was dispersed in purified water and administered at a dose volume of 5 mL/kg. All animals surviving treatment were killed on Day 15 and subsequently underwent a full necropsy.

Two males and four females died following a single oral dose of M58307 at 265 mg/kg. Deaths occurred within half an hour of dosing. Ante-mortem clinical signs included dyspnoea, proneness, spasticity and tonic convulsions. Necropsy revealed dark or inflated lungs.

One female rat died following oral administration of M58307 at a dose level of 141 mg/kg. Death occurred within half an hour of dosing. Ante-mortem clinical observations included dyspnoea, proneness and spasticity, salivation and opisthotonus. No macroscopic changes were identified during necropsy of the decedent.

Principal clinical signs of reaction to treatment at 265 or 141 mg/kg included dyspnoea, proneness, tonic convulsions, spasticity, ataxia, pilo-erection and tremors. Less commonly observed were signs of tachypnoea, lethargy, hunched posture, paddling motions of the paws, salivation, staining of the snout or anogenital soiling. Irritability and increased activity were apparent for some animals, generally noted after the first series of clinical signs. In addition, one of the males was noted to be gnawing the cage bars during the latter part of Day 1 and one female dosed at 141 mg/kg was biting the caging on Day 2. Recovery of surviving rats, as judged by external appearance and behaviour, was complete by Day 3 (141 mg/kg) or Day 6 (265 mg/kg).

No notable effects on bodyweight gain were recorded for any of the surviving rats dosed at 265 or 141 mg/kg. No macroscopic changes, considered to be attributable to treatment with M58307 at 265 or 141 mg/kg, were apparent at necropsy.

The approximate minimum lethal oral dose of M58307 to rats was found to be in the region of 141 mg/kg.

RESULTS

Preliminary study (Tables 1, 2, 3 and 4)

Deaths occurred among rats dosed in the preliminary phase at doses of 159 mg/kg or above. There was no consistent pattern to indicate a clear dose relationship to the mortality figures obtained. Two males died following dosing at 194 mg/kg but the male dosed at 212 mg/kg survived. One male died in each group dosed at 229 or 282 mg/kg but no males died in the groups dosed at 318 or 353 mg/kg. A similar pattern was noted among the females – one dosed at 159 mg/kg died but animals dosed at 194, 212, 229 or 282 mg/kg survived. All females dosed at 318 or 353 mg/kg died. The random mortality pattern seen in both males and females and the rapidity with which death occurred (generally within 30 minutes of dosing) suggested that simple dose-related toxicity was not the primary cause of death. The cause of death appeared to be more dependent upon the induction and severity of convulsive episodes but, as with mortality, no direct dose relationship was established.

The majority of clinical signs developed within the first hour following dosing for all of the preliminary groups and generally included the neuromuscular and breathing type changes. These typically included tonic convulsions, proneness, spasticity, twitching, ataxia, clonic convulsions, lethargy, tremors, paddling, abasia, arched or hunched posture and loss of movement due to limb rigidity and a stereotypic behavioural response which resulted in the animal biting the cage bars. The disruption to the animals breathing, probably resulting from convulsive episodes, was indicated by observation of tachypnoea, dyspnoea and/or hyperpnoea. Some of these signs were present, in varying degrees and at various group incidence, for animals dosed from 71 through to 353 mg/kg.

In addition, there were a number of other clinical signs that developed later in the observation period or were observed more sporadically or were considered to be of less clinical significance. Such signs included pilo-erection, salivation, staining of the snout or anogenital region, palpebral closure and exophthalmus. Later in the study, from Day 2 onwards some of the animals showed signs of increased activity, aggression and irritability. Clinical signs were relatively short-lived – animals dosed at 71 or 141 mg/kg had recovered by Day 2 and animals in the remaining groups had generally recovered by Day 3 to Day 5.

All of the rats dosed in the preliminary phase gained weight following dosing, weight gains were recorded at both the Day 1 to Day 4 and Day 4 to Day 8 intervals. The small weight losses apparent between Day −1 and Day 1 were wholly attributable to the period of pre-dose fasting. There was no evidence to indicate that administration of M58307 in the range of dose levels used in the preliminary phase (71 – 353 mg/kg) had any adverse effect on body weight gain.

No macroscopic changes were apparent for the majority of the preliminary group rats at necropsy either at the time of death or when killed on Day 8. Isolated changes were apparent among rats dosed at 194 or 318 mg/kg. Such changes included dark livers for the two decedent males dosed at 194 mg/kg and red lungs for one of these animals; an enlarged adrenal gland and a small ovarian cyst were apparent for one female dosed at 194 mg/kg and this animal was also missing the left kidney and the left horn of the uterus; the adrenals for the second female in this group were damaged. The stomach of one male dosed at 318 mg/kg had a yellow mucosal surface. None of the macroscopic changes noted showed any dose relationship nor any trend to help identify any particular target organ.

Main study - Mortality (Table 5)

There were deaths following a single oral dose of M58307 among rats of both sexes dosed at 265 mg/kg. Deaths occurred within half an hour of dosing. Two males and four females died. Since death was relatively rapid there were no notable changes in bodyweight between the pre-dose values and those recorded prior to necropsy.

Ante-mortem clinical signs included dyspnoea, proneness and spasticity in two of the males and tonic convulsions for one of these rats. Three of the female decedents died rapidly before clinical signs developed. The fourth female decedent showed the same signs as the males - dyspnoea, proneness and spasticity.

Necropsy of rats that died revealed only a degree of darkening of the lungs or inflation of the lungs both of which were probably agonal changes unrelated to administration of the test material and insufficiently severe to be considered causative for the death of these rats.

A single female rat dosed at 141 mg/kg died following oral administration of M58307. Death occurred within half an hour of dosing. The recorded weight for this animal showed no marked difference between pre-and post-dosing values.

Ante-mortem clinical observations included dyspnoea, proneness and spasticity, in common with other decedents, and additionally the female that died was salivating and had an episode of opisthotonus (a tonic convulsion involving rigid arching of the torso and head) prior to death.

No macroscopic changes were apparent during necropsy of the decedent.

Main study - Clinical signs (Table 6)

Initial clinical signs commonly observed among rats dosed at 265 mg/kg, generally apparent in the first 1-2 hours after dosing, included dyspnoea, proneness, tonic convulsions, spasticity and among the males, ataxia and tremors. Later on Day 1 or at later points during the observation period there were cases of salivation, pilo-erection, lethargy, staining of the snout, a hunched posture, irritability and increased activity. In addition, one of the males was noted to be gnawing the cage bars during the latter part of Day 1.

Recovery of surviving rats in this group, as judged by external appearance and behaviour, was complete by Day 6.

Initially the pattern of clinical signs observed among the rats dosed at 141 mg/kg were similar to those in the group dosed at 265 mg/kg namely dyspnoea, proneness, tonic convulsions, spasticity, ataxia, tremors and paddling motions of the paws. In addition there were cases, on Day 1, of pilo-erection, tachypnoea, lethargy, hunched posture, salivation and anogenital soiling. On Day 2 one of the females was irritable and showed increased activity.

Recovery of surviving rats in this group, as judged by external appearance and behaviour, was complete by Day 3.

Main study - Body weights (Table 7)

Among surviving rats from the group dosed at 265 mg/kg there were no notable effects on bodyweight. One male lost a small amount between the time of dosing and Day 2 but subsequently this and all other rats gained weight throughout the observation period.

All surviving rats dosed at 141 mg/kg gained weight during the first and second weeks of the observation period.

Main study - Necropsy findings (Table 8)

No macroscopic changes were observed on Day 15 for the animals dosed at 265 mg/kg.

No macroscopic changes were apparent for most of the rats killed on Day 15 following dosing at 141 mg/kg. The isolated change that was apparent, renal pelvic dilatation, is commonly observed as a background finding in males of this age and strain and was not considered to be consistent with a treatment related effect.

CONCLUSION

The mortality pattern established in this study, based on preliminary and main study findings, indicated that doses of 141 mg/kg or greater were likely to result in the death of rats. However there were no clear trends discernible to indicate dose relationships for death or particular clinical changes. Clinical signs were evident among the rats treated with the lowest dose level, 71 mg/kg, and consequently a no effect level was not established in this study, although the stated objectives of the protocol, namely approximation of the minimal lethal dose, were met.

The main study established that the approximate minimum lethal dose level of M58307 in the rat was approximately 141 mg/kg.