Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Magnesium metaborate

EC Number: 237-235-5

CAS Number: 13703-82-7

Index Number: -

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Not evaluated in this CLH proposal.

3.2 Acute toxicity - dermal route

Not evaluated in this CLH proposal.

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Not evaluated in this CLH proposal.

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Not evaluated in this CLH proposal.

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Not evaluated in this CLH proposal.

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Not evaluated in this CLH proposal.

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Not evaluated in this CLH proposal.

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Not evaluated in this CLH proposal.

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Not evaluated in this CLH proposal.

3.10 Reproductive toxicity

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3.10.1.1 Study 1

Study reference: [*Anon., 2017. Study report]*

Detailed study summary and results:

Test type

OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test). GLP compliance. Minor deviations, but considered not to affect the quality or integrity of the study.

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier
- Degree of purity, impurities and batch number not described

Test animals

- Rat/Sprague Dawley (Crl:CD(SD))/male and female
- 15 rats/sex/group in control and high dose (300 mg/kg bw/day) and 10 rats/sex/group for the doses 15, 50 and 125 mg/kg bw/day
- Age at study initiation: ~ 10 weeks
- Weight at study initiation: male: 310 g to 402 g, female: 195 g to 249 g

Administration/exposure

- Route of administration - oral (gavage)
- Duration of exposure: males dosed once daily for 28 days, females dosed once daily from study day 0 through the day prior to euthanasia (14 days prior to pairing through Lactation Day 13) for a total of 49 to 54 doses. 5 animals/sex in the control and high-dose group was subject to a 14-15 days recovery period.
- Doses: 0, 15, 50, 125, 300 mg/kg/bw/day. The dosing was based on the results of a 13-day range-finding study where the substance was administered to rats at the doses 100, 300, 500 and 1000 mg/kg bw/day.
- Control group was administered the vehicle arachis (peanut) oil

- Histirical control data available from the supplier Charles River
- Vehicle: arachis (peanut) oil, concentration in vehicle: 0, 1.5, 5, 12.5, 30 mg/mL, dose volume: 10 mL/kg
- Preparation of dosing solutions: The control and test substance formulations were prepared approximately weekly as single formulations for each dosage level, divided into aliquots for daily dispensation, and stored at room temperature, protected from light. On each day of dosing, aliquots of the control and test substance formulations were heated and stirred in a water bath set at approximately 50°C for a minimum of 30 minutes prior to dispensing and continuously throughout dosing. The control and test substance formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures. The first test substance dosing formulations were visually inspected by the Study Director and were found to be visibly homogeneous and acceptable for administration.
- Analytical verification of concentrations was performed
- Homogeneity and stability of the test substance in the vehicle following 7 days of room temperature storage at concentrations ranging from 10.0 to 100 mg/mL were established in a previous study. Therefore, stability assessments were not conducted in the current study. Samples for homogeneity and/or concentration determination were collected from the top, middle, and bottom strata of the first and 7th test substance dosing formulations and from the middle stratum of the first and 7th control group dosing formulations. One set of samples from each collection was subjected to the appropriate analyses. All remaining samples were stored at room temperature as back-up. All analyses were conducted by the Charles River Analytical Chemistry Department using a validated high performance liquid chromatography method with charged aerosol detection.

Description of test design:

- Details on mating procedure: The 10 rats/sex/group selected for evaluation of reproductive toxicity were paired on a 1:1 basis within each treatment group following 14 days of treatment for the males and females. A breeding record containing the male and female identification numbers and the start date of cohabitation was maintained. Each female was cohabitated with 1 male in a solid-bottom cage containing bedding material. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm following a vaginal lavage and verified by a second biologist. Each mating pair was examined daily. The day when evidence of mating was identified was termed gestation day 0. If evidence of copulation was not detected after 14 days of pairing, any females that had not shown evidence of mating were placed in solid-bottom cages. For the purpose of calculating pre-coital intervals, rats paired over a 12-hour dark cycle were considered to have been paired for 1 day.
- Mating, fertility, and copulation/conception indices were calculated as follows:

- Male (Female) Mating Index (%) = No. of Males (Females) with Evidence of Mating (or Confirmed Pregnant) / Total No. of Males (Females) Used for Mating x 100
- Male Fertility Index (%) = No. of Males Siring a Litter / Total No. of Males Used for Mating x 100
- Male Copulation Index (%) = No. of Males Siring a Litter / No. of Males with Evidence of Mating (or Females with Confirmed Pregnancy) x 100
- Female Fertility Index (%) = No. of Females with Confirmed Pregnancy / No. of Females with Evidence of Mating (or confirmed Pregnancy) x 100
- Female Conception Index (%) = No. of Females with Confirmed Pregnancy / No. of Females with Evidence of Mating (or Confirmed Pregnancy) x 100

- Parturition: All females selected for pairing were allowed to deliver naturally and rear their young to PND 13. During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. On the day parturition was initiated (PND 0), pups were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. Individual gestation length was calculated using the date delivery started.
- Premating exposure period for males and females (F0) was 14 days
- Dosing schedules and pre and post dosing observation periods for P: Animals were dosed once daily. No information on observation period in connection to the dosing.
- Parameters assessed for F0: Detailed clinical observations (twice daily: once in the morning, once in the afternoon), body weight, food consumption and compound intake, reproductive indices, haematology, clinical chemistry, neurobehavioural examination, immunology (thyroid hormone analysis), post-mortem examination (gross necropsy, histopathology, organ weights).
- Oestrous cyclicity (F0): Vaginal lavages were performed daily and the slides were evaluated microscopically to determine the stage of the oestrous cycle of each female for 10 days prior to test substance administration and continuing until evidence of copulation was observed (females selected for the breeding phase) or until termination of the mating period (females with no evidence of mating and recovery phase females) and for all females on the day o the scheduled necropsy. The average cycle length was calculated and reported for complete oestrous cycles (i.e., the total number of returns to metoestrus [M] or dioestrus [D] from oestrus [E] or prooestrus [P] until the detection of evidence of mating), beginning with the first day of dose administration. Oestrous cycle length was determined by counting the number of days from the first M or D in a cycle to the first M or D in a subsequent cycle. For breeding phase females, the cycle during which evidence of mating was observed for a given animal was not included in the mean individual oestrous cycle length calculation.
- Sperm parameters evaluated (F0): Histology and microscopic examinations of target tissues of testis, epididymis and sternal bone marrow (males only) were also examined from all F0 animals in all groups.

- Standardization of litters was performed on day 4 postpartum: To reduce variability among the litters, 8 pups per litter, 4 per sex when possible, were randomly selected on PND 4. Standardization of litter size was not performed on litters with fewer than 8 pups. All selections were performed by computerized randomization. Blood samples for possible future thyroid hormone analysis were collected from 2 culled pups/litter (pooled by litter) on PND 4; pup were euthanized by an intraperitoneal injection of sodium pentobarbital following blood collection and discarded. Remaining culled pups (not used for blood collection) were weighed, euthanized by an intraperitoneal injection of sodium pentobarbital on PND 4, and discarded.
- Parameters assessed for in F1: number and sex of pups, stillbirths, live births, postnatal mortality, offspring viability indices, presence of gross anomalies, weight gain, physical or behavioural abnormalities, anogenital distance (AGD), presence of nipples/areolae in male pups, thyroid hormone analysis, gross examination of dead pups (external and internal abnormalities) and gross necropsy (external and internal examinations including the cervical, thoracic, and abdominal viscera).
- Clinical observations performed in F1 and organs examined at necropsy in F1: not specified but performed.

Statistics

Results and discussion for F0 generation

Clinical signs

Test substance-related clinical observations of clear and/or red material around the mouth and red material around the nose were noted for F0 males and females in the 50, 125, and 300 mg/kg/day groups approximately 1 hour following dose administration generally throughout the dosing period. Although these observations were considered test substance-related, they generally did not persist to the daily examinations or detailed physical examinations and were not considered adverse.

Other clinical observations noted in the 15, 50, 125, and 300 mg/kg/day groups at the daily examinations, weekly detailed physical examinations, and 1 hour following dose administration, including hair loss on various body surfaces, cool body, and cool extremities, were noted infrequently and/or in a manner that was not dose-related.

Mortality

One female in the control group was found dead on Study Day 17. No remarkable clinical observations were noted for this female prior to death. The cause of death was attributed to acute pulmonary inflammation and additional pulmonary findings consisted of edema, hemorrhage, vascular inflammation, and thrombosis. The

inciting cause of pulmonary changes was undetermined, but gavage error could not be excluded. All remaining F0 males and females survived to the scheduled necropsies.

Body weight and body weight gain

Males:

- 300 mg/kg bw/day: test substance-related lower mean body weight gains were noted throughout the entire study (Study Days 0-28); the differences being generally significant ($p<0.01$) compared to the control group. Consequently mean body weights were significantly ($p<0.01$) lower (7.4% to 13.7%) than the control group from Study Days 13 through 27. Although a higher mean body weight gain in this group compared to the control group during the recovery period (Study Days 28 to 41) that were generally significantly ($p<0.05$ or $p<0.01$), mean body weights in this group remained 9.7% to 15.5% lower than the control group at the end of the study.
- 125 mg/kg bw/day: no difference in body weight or body weight gain compared to the control group.
- 50 mg/kg bw/day: no difference in body weight or body weight gain compared to the control group.
- 15 mg/kg bw/day: no difference in body weight or body weight gain compared to the control group.

Females

- 300 mg/kg bw/day: test substance-related lower mean body weight gains were observed during gestation. The differences from the control group was significant ($p<0.01$) during gestation days 7 to 11, 14 to 17, 17 to 20, and for the overall gestation period (days 0 to 20). Mean body weights were 4.0% to 21.7% lower than the control group during gestation; the differences were significant ($p<0.05$ or $p<0.01$) on gestation days 14, 17, and 20. Evaluation of mean body weights and body weight gains during lactation was precluded by euthanasia of females that delivered by Lactation Day 2
- 125 mg/kg bw/day: no difference in body weight or body weight gain compared to the control group during gestation. During lactation differences from the control group were transient, not statistically significant, and/or had no effect on the overall lactation interval.
- 50 mg/kg bw/day: no difference in body weight or body weight gain compared to the control group during gestation. During lactation differences from the control group were transient, not statistically significant, and/or had no effect on the overall lactation interval.
- 15 mg/kg bw/day: no difference in body weight or body weight gain compared to the control group during gestation. During lactation differences from the control group were transient, not statistically significant, and/or had no effect on the overall lactation interval.

Food consumption

Males:

- 300 mg/kg bw/day: substance-related lower mean food consumption occurred throughout the pre-mating period (Study Days 0 to 13). The differences were significant ($p<0.01$) compared to the control group. During the recovery period, mean food consumption in the 300 mg/kg/day group F0 males was similar to the control group.
- 125 mg/kg bw/day: Mean food consumption was similar to that in the control group throughout the study (no statistically significant differences).
- 50 mg/kg bw/day: Mean food consumption was similar to that in the control group throughout the study (no statistically significant differences).
- 15 mg/kg bw/day: Mean food consumption was similar to that in the control group throughout the study (no statistically significant differences).

Females

- 300 mg/kg bw/day: substance-related lower mean food consumption occurred during the pre-mating period (Study Days 0-13). Differences from the control group were significant ($p<0.01$) during Study Days 7-13. Mean food consumption in the females not paired for mating was similar to the control group during Study Days 13 to 48; differences were slight and not statistically significant. During the recovery period (Study Days 49-61), mean food consumption in the 300 mg/kg/day group females was similar or slightly higher than the control group. During gestation, mean food consumption was unaffected by test substance administration. During lactation, mean food consumption was precluded by euthanasia of females that delivered by Lactation Day 2.
- 125 mg/kg bw/day: significantly ($p<0.05$) lower mean food consumption during Study Days 7-13. However, in the absence of corresponding effects on mean body weights during this interval this difference was not considered adverse. no difference in food consumption compared to the control group during gestation or lactation.
- 50 mg/kg bw/day: no difference in food consumption compared to the control group during pre-mating, gestation or lactation.
- 15 mg/kg bw/day: no difference in food consumption compared to the control group during pre-mating, gestation or lactation.

Haematological findings

Effects observed considered non-treatment-related.

Clinical biochemistry

Males:

- 300 mg/kg bw/day: statistically significantly lower mean serum protein and lipid values and higher mean serum total bilirubin. The higher mean serum bilirubin value was due to 1 higher individual

value with a microscopic correlate of hepatocellular vacuolation. Other serum liver-specific parameters were similar to the control groups. The lower mean serum total protein, albumin, globulin, albumin/globulin (A/G) ratio, and cholesterol values were considered to be attributed to test item-related stress response and associated lower feed intake/weight loss and not to be a direct toxic effect. Higher mean serum A/G ratio values were noted; measured protein values were similar to the control groups and the magnitude difference was minimal. Lower mean serum glucose values were noted. The findings were not noted at the primary necropsy, were of minimal magnitude difference.

- 125 mg/kg bw/day: No significant changes compared to controls.
- 50 mg/kg bw/day: No significant changes compared to controls.
- 15 mg/kg bw/day: No significant changes compared to controls.

Females

- 300 mg/kg bw/day: Higher mean serum A/G ratio values were noted; measured protein values were similar to the control groups and the magnitude difference was minimal. Lower mean serum alanine aminotransferase (ALT) values were noted. The findings were not noted at the primary necropsy, were of minimal magnitude difference, and in the case of lower ALT values, were in a direction of no known toxicologic importance.
- 125 mg/kg bw/day: higher mean serum phosphorus and bile acid values were noted at the primary necropsy. No test item-related effects on serum chemistry parameters at the recovery necropsy.
- 50 mg/kg bw/day: higher mean bile acid values were noted at the primary necropsy. No test item-related effects on serum chemistry parameters at the recovery necropsy.
- 15 mg/kg bw/day: No significant changes compared to controls.

Behaviour (functional findings)

No significant changes in treated animals versus control animals.

Immunological findings

No effects observed.

Organ weight findings including organ/body weight ratios

Treatment-related effects were observed [descriptive text contains information on functional behavioural parameters and lack information organ weights]

Histopathological findings

Testes

Test item-related microscopic findings were noted in the testes of the 300 mg/kg bw/day males at primary necropsy. Testicular tubular degeneration, similar in severity grades in left and right testes, was characterized by variable degeneration and loss of germ cells and multinucleated germ cell formation. Germ cell degeneration was characterized by shrunken, hypereosinophilic germ cells. Minimal severity grade was characterized by individual germ cell degeneration and segmental depletion primarily affecting spermatocytes and round spermatids; reduced elongatingspermatids; and retained step 19 spermatids in late stage tubules. Mild severity grade was characterized by more pronounced germ cell depletion accompanied by variable germ cell disorganization and exfoliation. Moderate severity grade was characterized by widespread, moderate depletion of spermatocytes and round and elongating spermatids, and moderate germ cell disorganization and exfoliation. Marked severity grade was characterized by generalized depletion of germ cells with scattered Sertoli cell-only tubules. Spermatogonia were present in all severity grades. At recovery, testicular findings were more pronounced in the 300 mg/kg/day group males when compared with males at the primary necropsy. Marked to severe tubular degeneration/atrophy, characterized by a preponderance of tubules lined almost exclusively by Sertoli cells with variable numbers of sloughed, degenerate germ cells, was noted in all 300 mg/kg/day group males.

Epididymis

Test item-related microscopic findings at the primary necropsy were noted in the epididymis of the 300 mg/kg bw/day group. Epididymal findings were bilateral with similar magnitude severity grades, and consisted of reduced luminal sperm admixed with cellular debris, compatible with sloughed, degenerate germ cells. Findings were most pronounced in the caput and corpus, with milder changes within the cauda. At recovery, epididymal findings were more pronounced in the 300 mg/kg/day group males when compared with males at the primary necropsy.

Ovaries

Ovarian follicular cysts, characterized by mildly ectatic follicles lined by flattened follicular cells, were noted in a single 300 mg/kg/day group female. The finding was attributed to spontaneous change and not considered to be test item-related.

Liver

Test item-related microscopic findings (hepatocellular vacuolation) at the primary necropsy were noted in the livers of the 50, 125, and 300 mg/kg/day group males and a single 300 mg/kg/day group female. The hepatocellular vacuolation was characterized by discrete, variably-sized, vacuoles with well-defined margins in a centrilobular to diffuse distribution. The finding correlated with pale liver macroscopically.

Thymus

Microscopic findings consisted of reduced thymic cortical lymphocytes in the 15, 50, 125, and 300 mg/kg/day group males and females. The thymic change was characterized by thinning of the cortex accompanied in some animals by increased lymphocyte apoptosis.

Bone marrow

Decreased sternal bone marrow cellularity was observed in the 300 mg/kg/day group males. Reduced marrow cellularity was characterized by less densely organized progenitor cells and increased prominence of adipocytes.

Lungs

Thrombosis of large caliber pulmonary vessels with congestion of surrounding parenchyma was noted in the single unscheduled death control group female and in the 15 and 300 mg/kg/day group males and 15, 50, and 125 mg/kg/day group females. Thrombosis was accompanied by congestion and occasionally, vascular inflammation. Associated pulmonary congestion correlated macroscopically with dark red discoloration/areas in the lungs. The relationship between pulmonary thrombosis and administration of the test item was considered uncertain because of absence of the finding in the 300 mg/kg/day group females, presence of the finding in the single unscheduled death control female, absence of cardiovascular changes in any other tissues evaluated, and the findings were incompatible with the mode of action of the test item.

Reproductive function

Females

Significantly ($p<0.01$) longer mean gestation lengths were noted for the 125 and 300 mg/kg/day groups (22.0 and 22.5 days, respectively) compared to the control group (21.3 days). In the 300 mg/kg/day group, 3 of the 6 females that delivered had gestation lengths of 23 days and in conjunction with the test substance-related effects on intrauterine and postnatal survival in this group, the longer gestation length was attributed to the test substance. The value in the 125 mg/kg/day group was within the Charles River Ashland historical control data range (20.9 to 22.1 days) and in the absence of similar effects on pre- and postnatal survival, the effect at 125 mg/kg/day was not considered test substance-related. Mean gestation lengths in the 15 and 50 mg/kg/day groups were similar to those in the control group. No statistically significant differences were noted. No signs of dystocia were noted at any dosage level.

Males

Small epididymides noted macroscopically correlated with lower epididymal weights and bilateral reduced luminal sperm, and small, soft testes correlated with lower testicular weights and bilateral tubular degeneration. Testicular and epididymal degenerative changes were more pronounced at recovery. Findings were considered to be a direct toxicologic effect of the test substance, and were considered to be adverse in the 300 mg/kg/day group males.

Reproductive performance

No test substance-related effects on reproductive performance were observed at any dosage level. No statistically significant differences were noted between the control and test substance-treated groups. All females in all groups were gravid. The mean numbers of days between pairing and coitus in the test substance-treated groups were similar to the control group value. The mean lengths of oestrous cycles in these groups were also similar to the control group value. None of these differences were statistically significant.

Results and discussion for F1 generation

PND 0 litter data and postnatal survival

- 300 mg/kg bw/day: A lower number of pups were born. This corresponded to a higher number of unaccounted-for sites, and consequently mean live litter size on PND 0 (6.0 and 2.8 pups, respectively) when compared to the control group (13.6 pups for both). The differences were significant ($p<0.01$). Significantly ($p<0.05$) lower postnatal survival was noted in this group from PND 0 to 1. A test substance-related increase in the number of pups found dead and missing (presumed cannibalized) was noted PND 0 to 2 and PND 0 to 7 compared to the control group. Further evaluation of postnatal survival in the 300 mg/kg/day group was precluded by the death/euthanasia of the pups.
- 125 mg/kg bw/day: Survival was slightly lower than the control group during PND 0 to 1, 1 to 4, and birth to PND 4. Although the values were within the Charles River Ashland historical control data and the differences from the control group were not statistically significant, the slightly lower postnatal survival observed in the 125 mg/kg/day group was considered test substance-related due to the increase in the number of pups found dead and missing which was consistent with the test substance-related effects observed at 300 mg/kg/day and considered to be a dose-response effect.
- 50 mg/kg bw/day: Postnatal survival was unaffected by the test substance. The mean number of pups born, live litter size and the percentage of males at birth was similar to the control group values.
- 15 mg/kg bw/day: Postnatal survival was unaffected by the test substance. The mean number of pups born, live litter size and the percentage of males at birth was similar to the control group values.

Body weight and body weight changes

- 300 mg/kg bw/day: Evaluation of F1 body weights was precluded by the death or euthanasia of the majority of pups on PND 0 or 1. The pup body weight of the single pup in the 300 mg/kg/day group that was alive on PND 1 was 29.4% lower than the control group.
- 125 mg/kg bw/day: Mean pup birth weights (PND 1) were similar to the control group. However, test substance-related lower mean F1 male and female pup body weight gains were noted compared

to the control group during PND 1 to 4 and 4 to 7; the differences were generally significant ($p<0.05$). Mean body weight gains in this group were similar to the control group during PND 7 to 10 and 10 to 13. As a result of the lower body weight gains during the first week of the postnatal period, mean F1 male and female pup body weights in the 125 mg/kg/day group were lower (up to 15.0% and 13.5% for males and females, respectively) than the control group during PND 4 to 13; the greatest deficit was observed on PND 7.

- 50 mg/kg bw/day: mean F1 male and female pup body weight gains were similar to the control group during PND 1 to 4 followed by test substance-related lower mean body weight gains during PND 4 to 7; the differences were significant ($p<0.05$) compared to the control group during PND 4 to 7. The lower mean body weight gains observed at 50 mg/kg/day were not considered adverse because there were no test substance-related effects on postnatal survival noted at this dosage level. Mean body weight gains in this group were similar to the control group during PND 7 to 10 and 10 to 13. Mean F1 male and female pup body weights in the 50 mg/kg/day group were up to 13.2% and 10.9% lower for males and females, respectively, than the control during PND 4 to 13; the greatest deficit was observed on PND 7.
- 15 mg/kg bw/day: Mean F1 male and female pup body weights and body weight changes during PND 1 to 13 were unaffected by parental administration of the test substance. No statistically significant differences from the control group were noted.

Organ weights

Evaluation of organ weights in the 300 mg/kg/day group was precluded by the death/euthanasia of all pups in this group by PND 2. There were no test substance-related effects on thyroid weights in the F1 males and females at any dosage level on PND 13. Differences from the control group were considered to be the result of normal biological variation and were not considered to be of toxicological significance.

Gross pathological findings

Scheduled Pup Necropsies (PND 13)

In the 300 mg/kg/day group, PND 13 necropsy evaluations were precluded by the death/euthanasia of all pups in this group by PND 2. No internal findings that could be attributed to parental test substance administration were noted at the necropsy of pups euthanized on PND 13. One pup in the 125 mg/kg/day group was missing a tail. No internal findings were noted for pups at any dosage level.

Necropsies of Pups Found Dead

In the 300 mg/kg/day group the following malformation were found: 2 pups with anasarca, 1 pup with cleft palate, 2 pups with hydrocephaly, 3 pups with microphthalmia, 1 pup with only 12 pairs of ribs present, 1 had a right-sided aortic arch (the aortic arch and descending aorta coursed to the right of the vertebral column, right carotid and subclavian arteries arose independently from the aortic arch [no brachiocephalic

arch]; left carotid and subclavian arteries arose from a common vessel from the aortic arch), and 1 pup had sternoschisis (sternal band nos. 2 through 4 not joined). In addition, fetal developmental variations observed in fetuses in the 300 mg/kg/day group included renal papilla(e) not fully developed and/or distended ureter(s) for 2 pups and a major blood vessel variation (right carotid and subclavian arteries arose independently from the aortic arch [no brachiocephalic trunk] for another pup. No foetal malformations or developmental variations were noted for pups that were found dead in the 15, 50, or 125 mg/kg/day groups.

Anogenital distance

Evaluation of anogenital distance in the 300 mg/kg/day group was precluded by the death or euthanasia of the majority of pups on PND 0 or 1. The anogenital distance (2.51 mm) of the single pup in this group that survived until PND 2 was smaller than the mean control group value (3.68 mm). The anogenital distances (absolute, relative to pup body weight, and relative to the cube root of pup body weight) in the 15, 50, and 125 mg/kg/day groups were similar to the control group values. Differences from the control group were slight and not statistically significant.

Areolae/Nipple Anlagen

Evaluation of areolae/nipple anlagen in the 300 mg/kg/day group was precluded by the death/euthanasia of all pups in this group by PND 2. Areolae/nipple anlagen in the F1 male pups was unaffected by parental administration of the test substance when evaluated on PND 13. The test substance-treated group values were not statistically different from the control group values.

3.11 Specific target organ toxicity – single exposure

Not evaluated in this CLH proposal.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 [Study 1]

Study reference:

[Anon., 2016. Study report]

Detailed study summary and results:

Test type

13-Day Oral (Gavage) Toxicity Study in Rats (according to WIL Research SOPs and the study protocol as approved by the Sponsor). Non-GLP. Limit test.

The objective of the study was to determine dosage levels of magnesium metaborate to be evaluated in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422) in rats.

Test substance

- Magnesium metaborate
- EC number: 237-235-5
- CAS number: 13703-82-7
- Degree of purity: not specified
- Impurities: not specified
- Batch number: not specified

Test animals

- Rat, Sprague-Dawley (Crl:CD(SD))
- 5 rats/sex/group
- Age at study initiation: 11 weeks
- Weight at the study initiation: Male body weights ranged from 346 to 404 g and female body weights ranged from 222 to 258 g on study day 0.

Administration/exposure

- Route of administration: oral gavage
- Duration and frequency of test/exposure: One daily dosing for 13 days
- Doses: 0, 100, 300, 500, 1000 mg/kg bw/day. The doses were set to determine the dosage levels for the subsequent combined repeated dose toxicity study with reproduction/developmental toxicity screening (OECD 422) with 1000 mg/kg bw/day being the limit dose.
- Vehicle:arachis oil. Dose volume: 10 mL.
- The control and test substance formulations were prepared twice as single formulations for each dosage level, divided into aliquots for daily dispensation, and stored at room temperature, protected from light. On each day of dosing, aliquots of the control and test substance formulations were heated and stirred in a water bath set at 50°C ± 5°C for a minimum of 30 minutes prior to dispensing and continuously throughout dosing. The control and test substance formulations were stirred continuously throughout the preparation and dose administration procedures.

Examinations

Observations and examinations performed and frequency

- Clinical observations: All rats were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded daily (prior to dose administration during the treatment period). Animals were also observed for signs of toxicity approximately 1 hour following dose administration. The absence or presence of findings was recorded for all animals. In addition, the presence of findings at the time of dose administration was recorded for individual animals.
- Body weight: Individual body weights were recorded on study days 0, 4, 7, 11, and 13. Mean body weights and body weight changes are presented for each interval. In addition, mean body weight changes were calculated for the overall treatment period (study days 0-13). When body weights could not be determined for an animal during a given interval (due to an unscheduled death, weighing error, etc.), group mean values were calculated for that interval using the available data.
- Food consumption for each animal was recorded on study days 0, 4, 7, 11, and 13. Food consumption was calculated on a per cage basis for the corresponding body weight intervals. Food consumption was normalized to the number of animals/cage and was reported in g/animal/day.
- Gross necropsy was performed on animals that were found dead or euthanized (by carbon dioxide inhalation) in extremis or due to early group termination during the course of the study. Necropsy included examination of the cranial, thoracic, abdominal, and pelvic cavities. Tissues were preserved in 10% neutral-buffered formalin only as indicated by the gross findings. The carcasses were then discarded. Gross necropsy was also conducted on all surviving animals at the scheduled euthanasia on study day 13. All animals were euthanized by carbon dioxide inhalation. Necropsy included examination of the external surface, all orifices, the external surface of the brain, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. Tissues were preserved in 10% neutral-buffered formalin only as deemed necessary by the gross findings. All carcasses were then discarded. The following organs were weighed from all animals at the scheduled necropsy:
  - Adrenal glands
  - Liver
  - Brain
  - Ovaries with oviducts
  - Epididymides
  - Spleen
  - Heart
  - Testes
  - Kidneys
  - Thymus gland
  - Thyroids with parathyroids

  Except as noted, paired organs were weighed together. Absolute weights and organ to final body weight and organ to brain weight ratios were reported.

Results and discussion

Clinical signs

- 1000 mg/kg bw/day: Clinical observations in males and females included unkempt appearance, red material around the nose, yellow material around the urogenital and anogenital areas, flushed extremities, clear material around the mouth, and/or thin body at the daily examinations and/or approximately 1 hour following dose administration.
- 500 mg/kg bw/day: Increased incidences of red material around the nose and clear material around the mouth were noted for males and females group at the daily examinations and/or approximately 1 hour following dose administration. In addition, a single female in the 500 mg/kg/day group had a reddened facial area and/or ears at the daily examinations during study days 11-13.
- 300 mg/kg bw/day: No remarkable clinical observations were noted for males and females at the daily examinations or approximately 1 hour following dose administration.
- 100 mg/kg bw/day: No remarkable clinical observations were noted for males and females at the daily examinations or approximately 1 hour following dose administration.

Mortality

- 1000 mg/kg/day: 2 male rats were found dead on study day 4 and 5, respectively. In addition, 3 females in this group were euthanized in extremis on study day 4. Mean body weight losses (35 g to 54 g) and/or reduced food consumption (6 g/day) were noted for these animals prior to death/euthanasia. The remaining males and females were euthanized due to excessive toxicity on study day 5.
- 500 mg/kg bw/day: 1 male rat was euthanized in extremis on study day 8 following a body weight loss (48 g) and clinical observations that included vocalization during handling, impaired use of the hindlimbs, a body cool to the touch, unkempt appearance, red material around the nose and eye, and yellow and brown material around the urogenital and anogenital areas, respectively. All other males and females survived to the scheduled necropsy on study day 13.
- 300 mg/kg bw/day: All males and females survived to the scheduled necropsy on study day 13.
- 100 mg/kg bw/day: All males and females survived to the scheduled necropsy on study day 13.

Body weight and weight changes

- 1000 mg/kg bw/day: Mean body weight losses were noted for males and females during study days 0-4; the differences were significant ($p<0.01$) compared to the control group. Consequently, mean body weights in this group were 14.2% and 11.4% lower for males and females, respectively, than the control group on study day 4; the difference in males was significant ($p<0.01$). Further evaluation of body weight data in this group was precluded by early group termination on study day 5.

- 500 mg/kg bw/day: Mean body weight losses and lower mean body weight gains were noted for males during study days 0-11 and mean body weight losses were noted for females during study days 0-7. The differences were generally significant ($p<0.05$ or $p<0.01$) compared to the control group. As a result, mean body weight losses were noted for males and females in this group when the overall treatment period (study days 0-13) was evaluated; the difference in males was significant ($p<0.01$) compared to the control group. In addition, mean body weights in males and females were lower (up to 12.6% and 10.4%) than the control group during study days 7-13; the differences were significant ($p<0.05$ or $p<0.01$) for males on study days 7, 11, and 13 and for females on study day 7.
- 300 mg/kg bw/day: Lower mean body weight gains were noted for males during study days 4-13 and females generally throughout the treatment period (study days 0-13); none of the differences were statistically significant compared to the control group. Consequently, lower (not statistically significant) mean body weights gains were noted for males and females compared to the control group when the overall treatment period (study days 0-13) was evaluated and mean male and female body weights in this group were 5.6% and 7.7% lower than the control group on study day 13.
- 100 mg/kg bw/day: Mean body weights and body weight gains were similar to that in the control group. Differences were slight and not statistically significant.

Food consumption

- 1000 mg/kg bw/day: Mean food consumption in males and females was lower than the control group during study days 0-4. Further evaluation of food consumption data in this group was precluded due to early group termination on study day 5.
- 500 mg/kg bw/day: Mean food consumption was lower than the control group throughout the treatment period.
- 300 mg/kg bw/day: Mean food consumption was lower than the control group throughout the treatment period.
- 100 mg/kg bw/day: Mean food consumption was lower than the control group throughout the treatment period.

Organ weights

- 1000 mg/kg bw/day: Could not be evaluated due to the mortality in this group.
- 500 mg/kg bw/day: Significantly ($p<0.05$) lower mean liver weights (absolute and relative to brain weight) for males were noted compared to the control group. Lower mean thymus weights (absolute and relative to final body and brain weights) were noted for males and females; the differences were significant ($p<0.01$) compared to the control group.

- 300 mg/kg bw/day: Lower mean thymus weights (absolute and relative to final body and brain weights) were noted for males and females; the differences were significant ($p<0.01$) compared to the control group.
- 100 mg/kg bw/day: Lower mean thymus weights (absolute and relative to final body and brain weights) were noted for males; the differences were significant ($p<0.01$) compared to the control group.

Gross pathology

- 1000 mg/kg bw/day: All males and females were found dead or euthanized in extremis or due to early group termination by study day 5. Macroscopic findings observed in males and females included dark red discoloration of the adrenal glands, small spleen, small thymus, yellow and red matting of the skin, and/or red matting of the paws.
- 500 mg/kg bw/day: One male was euthanized in extremis on study day 8. Macroscopic findings for this male included yellow, brown, and red matting of the skin. At the scheduled necropsy on study day 13, macroscopic findings observed included dark red discoloration of the adrenal glands and small thymus for males and females and pale liver for females.
- 300 mg/kg bw/day: Dark red discoloration of the adrenal glands was noted for 2 males.
- 100 mg/kg bw/day: No significant pathological findings.

3.13 Aspiration hazard

Not evaluated in this CLH proposal.

4 ENVIRONMENTAL HAZARDS

Not evaluated in this CLH proposal.