Karstedt Concentrate

Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422 2015 version):

- Dose levels of 30, 125 and 500 mg/kg/day
- Included satellite animals dosed at 0 or 500 mg/kg/day for 28 days for blood sampling (TK and micronucleus testing). *In vivo* micronucleus assessment by Micro Flow method at Litron Laboratories, USA. Positive controls included (2 dose levels of mitomycin C or vincristine) for micronucleus.
- Included pre-treatment smears for animal selection, and thyroid hormone analysis
- Full draft report due 24 Mar 17

Karstedt - 422 study

500 mg/kg/day: males ↓ bw from D4 day (max -14.4%), ↓ food consumption both sexes week 1 only, salivation was noted in all males and 5/10 females. One female died on LD6.

Haematological differences: ↑ in white blood cell counts in both sexes (males: granulocytes, lymphocytes, monocytes, large unstained cells; females monocytes), ↑ reticulocytes in males.

Clinical chemistry, neurological screening – no effects

Organ weights: *males:* ↑ in relative organ weights for testes, kidneys, liver and epididymides; ↓ in absolute weight of spleen, thymus and prostate with seminal vesicles, all reflecting lower body weights; *females*: ↑ absolute and relative adrenal weight

Pathology: 5/10 males and 6/10 females showed macroscopic and/or microscopic abnormalities in the lung (both sexes) and adrenal (females only) and stomach.

Karstedt - 422 study (cont.)

500 mg/kg/day

Micropathology:

- Lungs: multiple granulomatous inflammation in combination with haemorrhages and pigment deposition. Occasional multi-nucleated giant cells indicating a foreign body response to the test item with associated granulomatous inflammation and haemorrhage with hemosiderin–laden macrophages (pigment deposition).
- Adrenals: hypertrophic adrenals in 3/6 females (N.B. group mean adrenal weight increased but no specific correlation on individual animal basis)

i.e. No evidence of specific target organ toxicity

Karstedt – 422 study (cont.)

Reproductive performance:

	Dose level (mg/kg bw/day)			
	0 control	30	125	500
Females paired	10	10	10	10
No of litters	9	10	8	9
Corpora lutea	14.9	16.1	15.1	16.0
Implantation sites	14.8	15.7	15.1	15.6
Pre-implantation loss (dam/group)	0.8 (0.9)	2.5 (2.1)	0.0 (0.0)	2.8 (2.3)
Post-implantation loss (dam/group)	3.8 (3.4)	9.6 (10.2*)	14.1 (13.8**)	15.1 (14.3**)
Mean pups/litter day 1 (total)	14.2	14.4	13.0	14.0
Mean pups/litter day 1 (alive)	14.2	14.2	13.0	13.2
Total dead pups day 1 (litters)	0	2 (1)	0	7 (3)
Mean pup body weight day 1	6.97	6.85	6.68	5.93**
Pups died LD1-4	2	3	1	32/17+
Viability index (to LD4); dam/group)	98.6 (98.4)	96.7 (96.5)	99.2 (99.0)	72.1 (73.1**)
Pups died LD1-13	3	3	2	32/17+
Mean pup body weight day 1	6.97	6.85	6.68	5.93**
Mean pup body weight day 4	10.22	10.03	9.92	8.42*
Mean pup body weight day 13	29.99	28.66	27.46	20.55**

+ excluding 15 pups from intercurrent female 77

Increased post-implantation loss at 30 and 125 considered spontaneous as generally within the range of historic control data

Karstedt – 422 study (cont)

Concerns for reproductive toxicity:

- Developmental: increased number of stillbirths, decreased viability index, increased no of post-implantation loss.
- Lactational effects: increased pup mortality, early onset of delayed pup growth

Both present with limited evidence of systemic toxicity

Karstedt – 422 study (cont.)

Micronucleus assay:

- No test item-related increase of micronucleated reticulocytes in males or females animals at 500 mg /kg bw/day as compared to the corresponding vehicle control group.
- The positive reference control groups which received Mitomycin C (0.75 mg/kg bw, i.p.) or Vincristin sulphate (0.04 mg/kg bw, i.p.) exhibited a statistically significant increase in the number of micronucleated reticulocytes demonstrating the validity of testing procedure.

Karstedt – 422 study (cont.)

Ano-genital distance, nipple retention in males

No effects at any dose level

Thyroid hormone analysis:

- Adult males: At 500 mg/kg bw/day T4 ↓ (53.67 v 65.05 nmol/L)
- Adult females (PND 14): No effects
- Pups:
  - PND 4 no effects
  - PND 13 ↓ in males (48.34 v 67.08 nmol/L) and females (56.36 v 68.81 nmol/L) at 500 mg/kg bw/day

Thyroid weight:

- Adult males: no effects
- Adult females: no effects
- Pups (PND 13): no effects

Thyroid histopathology:

No effects

Karstedt – 422 study (cont.)

Conclusion on Endocrine Effects

The lack of effect in pregnant females and in PND 4 pups indicates that reduction in thyroid hormones in fetuses and possible consequences on fetal brain development is unlikely. By PND 13, T4 was reduced in male and female pups at 500 mg/kg only, indicating that as the dosage of Karstedt Concentrate increased (via increased milk consumption) then this triggered a similar response to that seen in the F1 males. There was no accompanying increase in thyroid weight or histopathology in any of the affected groups, indicating only a modest effect on the thyroid hormonal system.

The thyroid hormone reduction maybe a secondary consequence of increases in liver weight (although increase not large, liver not weighed in pups).

Karstedt – 422 study (cont.)

Report conclusions:

125 mg/kg bw/day: No treatment-related effects

30 mg/kg bw/day: No treatment-related effects

NOAELs:

Systemic toxicity: 125 mg/kg bw/day (↓ bw in males, mortality in 1 female)

Reproductive toxicity:

Fertility and reproduction: 500 mg/kg bw/day

Pre-natal development: 125 mg/kg bw/day (↑ stillbirths, ↓ live born index)

Post-natal development: 125 mg/kg bw/day (↓ viability, ↓ pup bw)

Karstedt 422 (cont.)

Conclusions re classification:

STOT-RE: no evidence of significant target organ toxicity at any dose level. Not classified

REPRO: Cat 1B H360 May cause damage to unborn child

plus

H362 May cause harm to breast fed children

Ruthenium Chloride (1)

14-day dose range finding toxicity/palatability study in rats:

- Dietary inclusions at 3750, 7500 and 15000 ppm (eq ~ 311, 639, 1194 mg/kg/day)
- No significant toxicity at any dose level
- 15000 ppm was associated with lower body weight (~ 6% on day 13), body weight gain (-31.4% days 0-13) and reduced food consumption in males. No effects in females
- Report issued 1-Mar-17

Ruthenium Chloride (2)

28-day dietary toxicity study with 14 day recovery period (OECD 407):

- Dietary inclusions at 500, 1500, 5000, 15000 ppm (eq. 38/47, 114/147, 365/449, 1149/1326 mg/kg/day in males/females)
- Recovery groups included at 0, 5000 and 15000 ppm
- Clinical signs, FOB data, body weight, food consumption, macroscopic and microscopic findings, organ weights, haematology and clinical chemistry indicate no notable signs of significant treatment-related toxicity.
- Body weight slightly lower at 15000 ppm (5.4%/3.1% on day 27 but no effect after 14 day recovery period)
- NOAEL = 5000 ppm (407 mg/kg bw/day).

Ruthenium Chloride (3)

Reproduction/developmental toxicity screening test (dietary route) (OECD 421 2016 version):

- Dietary inclusions at 1500, 5000, 15000 ppm (93.6/152.9, 313/524,957.9/1593.7 mg/kg/day in males/females)
- No adverse treatment-related clinical signs at any dose level.
- Males at 15000 ppm ↓ bw (7.1%), ↓ food consumption (9%)
- No effects on reproductive performance, gestation, parturition
- No effects on pup survival, bw, growth
- No effects on ano-genital distance, nipple retention in males
- No toxicologically significant effects on organ weights
- No treatment-related macroscopic or microscopic findings

Ruthenium Chloride (4)

Summary table of the thyroid hormone analysis

Parameters	Groups/Concentration (mg/kg bw/day)			
	0	Low dose	Mid dose	High dose
Thyroxine (T4) level (nmol/L)				
Parental males	64.300	60.932	51.305	52.667
Parental females	43.456	43.450	41.183	36.067
PND 4 pups	28.118	29.498	26.115	25.773
PND 13 pups	84.591	80.845	70.550	63.575
Thyroid-Stimulating Hormone (TSH) level (nmol/L)				
Parental males	2.866	2.955	3.288	2.840
Parental females	2.744	2.779	3.601	3.141
PND 13 pups	below LOQ	below LOQ	below LOQ	below LOQ

Ruthenium Chloride (5)

Conclusions re classification:

STOT-RE: no evidence of significant target organ toxicity at any dose level. Not classified

REPRO: no evidence for effects on reproductive ability, fertility or lactation and development. Not classified