**Labeling Recommendations:**

[

]

**Addendum/appendix listing:**

Dose-ranging study report:
- Mouse: Page 40 of the initial pharmacology/toxicology review
- Rat: Pages 42 of initial pharmacology/toxicology review, Pages 70 and 76 of the review of carcinogenicity study.

CAC report: Page 95 of the review of carcinogenicity study.

Sponsor's incidence of histopathology findings:
- Mouse: Page 23 of the review of carcinogenicity study.
- Rat: Page 50 of the review of carcinogenicity study.

List of organs and tissues examined:
- Mouse: Page 17 of the review of carcinogenicity study.
- Rat: Page 44 of the review of carcinogenicity study.

Body weight changes versus dose level:
- Mouse: Page 19 of the review of carcinogenicity study.
- Rat: Page 46 of the review of carcinogenicity study.

## VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

**Study title: Oral administration prior to and in the early stages of pregnancy in rats**

Full review is attached on Page 48 of the initial pharmacology/toxicology review.

**Key study findings:** rats were treated with rosuvastatin at oral doses of 5, 15 and 50 mg/kg/ prior to and in the early stages of pregnancy.

1. No toxic symptoms nor death occurred in each sex in any groups. In females in the 50 mg/kg group, however, persistent suppression of the body weight gain (4% decrease compared with control) and sporadic decreases in the food consumption were observed during the treatment period.
2. S-4522 had no effect on the estrus cycle, copulation, male/female fertility, ovulation, implantation and maintenance of pregnancy.
3. S-4522 caused slight low fetal body weight and slightly retarded ossification at 50 mg/kg but had no embryo-fetolethal and teratogenic effect.
4. Based on these, NOAEL was estimated to be 15 mg/kg for parental animals in the aspect of general toxicology and 50 mg/kg/day in the aspect of reproductive toxicology and 15 mg/kg for embryos/fetuses.

**Study title: Oral administration during the period of organogenesis in rats**

Full review is attached on Page 51 of the initial pharmacology/toxicology review.

**Key study findings:** rats were treated with rosuvastatin orally at doses of 25, 50 and 100 mg/kg/day during the fetal organogenesis period.

1. Effects on dams: body weight gain decreased 4% with sporadic decrease in food consumption in the 100 mg/kg/day group, and the liver weight increased 6% and 12% in the 50 and 100 mg/kg/day groups, respectively. S-4522 had no effects on the establishment or duration of pregnancy, delivery and lactation conditions of the dams.
2. Effects on fetuses and offspring: S-4522 induced treatment related increases in visceral malformation (thymic remnant in neck) at $\geq$ 25 mg/kg, and skeletal variations (lumbar rib, asymmetry of sternebra in 2 fetuses) at 100 mg/kg in fetuses. It had no effects on the viability of the offspring, functional/behavioral development, learning ability or reproductive function of the offspring.
3. Based on these, the NOAEL was estimated to be 25 mg/kg/day for dams in the aspect of general toxicology and $\leq$ 25 mg/kg/day for maternal reproductive function, fetuses and offspring under the conditions tested.

**Study title: Pre- and post-natal developmental toxicity study in rat**

**Key study findings:** mated female Sprague Dawley rats were dosed once daily, by oral gavage, with ZD4522 at dose levels of 0, 0, 2, 10 or 50 mg/kg/day from day 7 of gestation, throughout pregnancy and until day 21 of lactation. No apparent treatment related changes were observed either in the dams or the offspring at dose levels of up to 10 mg/kg/day. Lower number of pups live born and incidence of pups with eyes open on day 16 post partum, and

increase in startle amplitude for males were noted in the 50 mg/kg. Renal toxicity was observed in dams at 50 mg/kg. Therefore, together with the previous study, the NOAEL was estimated to be 25 mg/kg/day for dams in the aspect of general toxicology and 10 mg/kg/day for maternal reproductive function, fetuses and offspring.

**Study no.:** RR0824
**Volume #, and page #:** electronic submission file name: twr2899.pdf
**Conducting laboratory and location:** ———

**Date of study initiation:** May 1999
**GLP compliance:** Yes
**QA reports:** yes (X ) no ( )
**Drug, lot #, radiolabel, and % purity:** A87002 / ADM 03516E98, Purity: 96.7%
**Formulation/vehicle:** 5% gum Arabic

**Methods:**

Species/strain: Rats, Sprague Dawley
Doses employed: 0, 0, 2, 10, and 50 mg/kg
Route of administration: oral gavage, 1 ml/100g
Study design: mated female Sprague Dawley rats were dosed once daily from day 7 of gestation, throughout pregnancy and until day 21 of lactation. The pups from the litters produced were individually identified on day 3 post partum and were allocated to the F1 study, but were not dosed with the test material.
Number/sex/group: 24
Parameters and endpoints evaluated: Clinical observations, bodyweight and food consumption were recorded for all parental females. On day 22 post partum, the dams were examined post mortem and the livers were removed, weighed and processed for microscopic examination. Clinical observations and bodyweight were recorded for the F1 generation. Additional F1 observations included pre-weaning developmental landmarks and post weaning functional observations. Vaginal opening and preputial separation were recorded for all offspring selected for rearing to sexual maturity. After reaching sexual maturity, the F1 generation females were paired with males from the same group. Following mating, the females were killed on day 13 of gestation, examined post mortem and the uterine contents examined. The males were examined post mortem after completion of the mating period.

**Results:**

Mortality: One female (#85) at 10 mg/kg was found dead on day 24 of gestation. No previous adverse clinical signs had been recorded for this animal. Histological examination of the uterus revealed haemorrhage, oedema and congestion which were consistent with the macroscopic uterine findings and suggest that uterine torsion was the cause of death. Another female (#111) at 50 mg/kg was killed for humane reasons on day 14 of gestation following signs including being subdued and cold, with pallor, piloerection, eye pallor and hunched posture. Histological examination of the organs revealed an atrial thrombosis in the heart and nephropathy and medullary tubular necrosis of the kidney.

Clinical signs: no treatment related change.

Body weight: no treatment related changes on maternal bodyweight during gestation or lactation.

FIGURE 1 - GROUP MEAN MATERNAL BODYWEIGHT DURING GESTATION

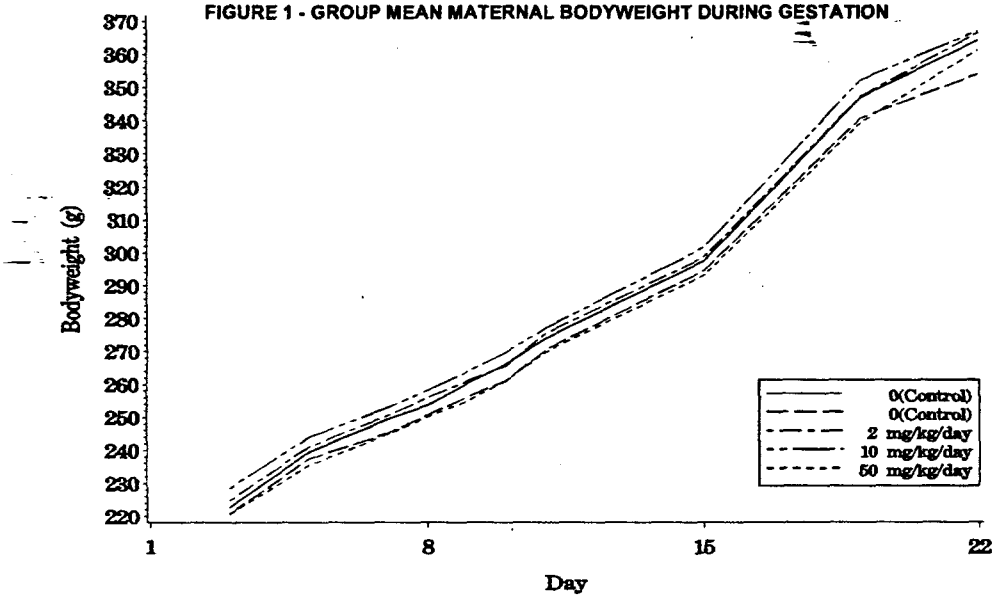
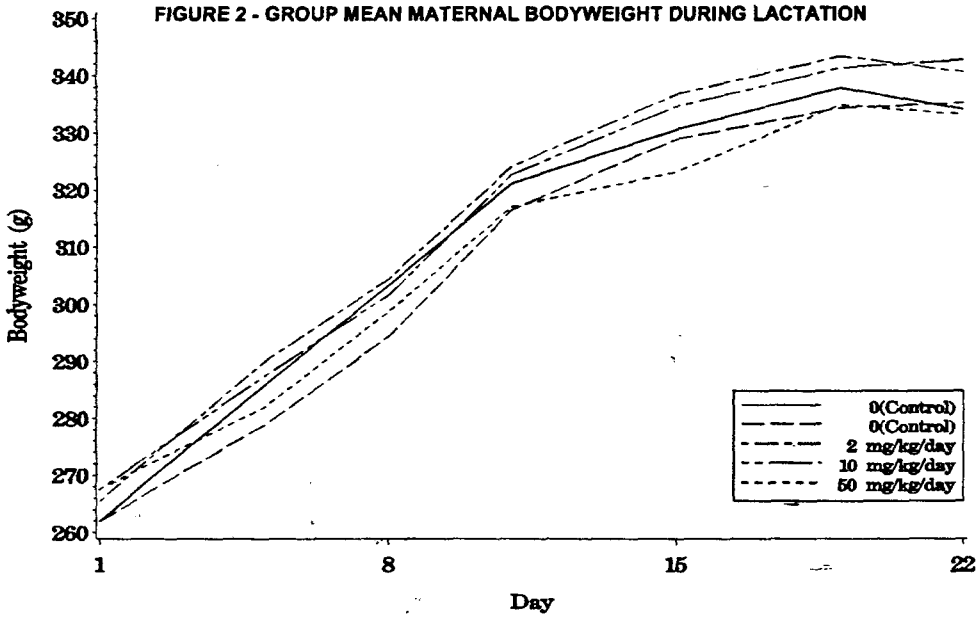



FIGURE 2 - GROUP MEAN MATERNAL BODYWEIGHT DURING LACTATION




Food consumption: no treatment related change.

Toxicokinetics: no data available.

**In-life observations:**

**Dams:** no apparent treatment related changes.

**Offspring**: the number of pups live born was statistically significantly lower at 50 mg/kg/day in comparison with the control group (89.9% in 50 mg/kg group vs. 98.6% in control), due to two dams with the non viable litters in 50 mg/kg group. There were statistically significant lower incidence of pups with eyes open on day 16 post partum in the 50 mg/kg/day group (74.6% - 75.6% in 50 mg/kg groups vs. 89.0% - 96.9% in controls); and significant increase in startle amplitude for males during reps 21 to 30 for both the 10 and 50 mg/kg/day groups. No apparent treatment related changes in fetal mortality beyond birth, fetal body weight, the timing of developmental landmarks such as pinna detachment, sexual maturity or learning and memory tests or motor activity. Reproductive performance was similar in all groups.

**INTERGROUP COMPARISON OF STARTLE AMPLITUDE (V) - MALES**

| DAY 23 | | Dose level of ZD4522 (mg/kg/day) 0(Control) | 0(Control) | 2 | 10 | 50 |
|---|---|---|---|---|---|---|
| Repetition 1-10 | MEAN | 161.1 | 146.9 | 141.1 | 162.5 | 168.4 |
| | S.D. | 87.9 | 91.8 | 85.1 | 83.5 | 86.3 |
| | N | 20 | 20 | 20 | 20 | 19 |
| Repetition 11-20 | MEAN | 142.2 | 111.9 | 109.2 | 144.5 | 140.6 |
| | S.D. | 81.6 | 83.2 | 83.2 | 79.5 | 87.8 |
| | N | 20 | 20 | 20 | 20 | 19 |
| Repetition 21-30 | MEAN | 101.7 | 92.7 | 113.5 | 140.8* | 150.1** |
| | S.D. | 49.9 | 79.9 | 80.5 | 71.5 | 77.6 |
| | N | 20 | 20 | 20 | 20 | 19 |
| Repetition 31-40 | MEAN | 104.7 | 99.8 | 106.4 | 136.7 | 126.4 |
| | S.D. | 55.1 | 86.1 | 85.1 | 70.0 | 72.7 |
| | N | 20 | 20 | 20 | 20 | 19 |
| Repetition 41-50 | MEAN | 104.7 | 107.8 | 114.4 | 123.3 | 128.5 |
| | S.D. | 54.3 | 76.5 | 85.6 | 67.5 | 76.7 |
| | N | 20 | 20 | 20 | 20 | 19 |

**Terminal and necroscopic evaluations:**

**Dams:** One female (number 85) at 10 mg/kg was found dead on day 24 of gestation. No previous adverse clinical signs had been recorded for this animal. Histological examination of the uterus revealed haemorrhage, oedema and congestion which were consistent with the macroscopic uterine findings and suggest that uterine torsion was the cause of death. One female (number 111) at 50 mg/kg was killed for humane reasons on day 14 of gestation following signs including being subdued and cold, with pallor, piloerection, eye pallor and hunched posture. Histological examination of the organs revealed an atrial thrombosis in the heart and nephropathy and medullary tubular necrosis of the kidney. One female at 50 mg/kg had renal findings including slight bilateral hydronephrosis, slight tubular degeneration/ necrosis, slight tubular dilatation, and slight nephropathy.

**Offspring:** no abnormalities observed.

**Summary of individual study findings:**

No apparent treatment related changes were observed either in the dams or the offspring following oral administration of ZD4522 to the dams from day 7 of gestation until day 21 post partum, at dose levels of up to 10 mg/kg/day. Lower number of pups live born and incidence of pups with eyes open on day 16 post partum, and increase in startle amplitude for males were noted in the 50 mg/kg. Renal toxicity was observed in dams at 50 mg/kg. Therefore, together with the previous study, the NOAEL was estimated to be 25 mg/kg/day for dams in the aspect of general toxicology and 10 mg/kg/day for maternal reproductive function, fetuses and offspring.

**Study title: Oral administration during the period of fetal organogenesis in rabbits**

Full review is attached on Page 56 of the initial pharmacology/toxicology review.

**Key study findings:** S-4522 was repeatedly administered orally at doses of 0.3, 1 and 3 mkd to groups of 17 copulated rabbits during the fetal organogenesis period of pregnancy.

1. Effect on dams:
   0.3 and 1 mkd groups: no significant changes observed.
   3 mkd group: 2 death and 2 killed moribund. Weight loss, food consumption decrease, abortion and histopathological changes in liver (vacuolation of the centrilobular hepatocyte), kidney (necrosis and mineralization (calcium deposition) of the cortical tubular epithelial cells), gallbladder (ulceration, hemorrhage or inflammatory cell infiltration of the mucosa), heart(necrosis and mineralization (calcium deposition) of the cardiac muscle fibers) and muscle (mineralization (calcium deposition) of the necrotic foci of the muscle fibers) were observed in these animals.
2. Effects on fetuses: No embryo-fetolethal effect nor any growth suppressant or teratogenic effect on fetuses were observed.
3. Conclusion: NOAEL is 1 mg/kg/day for dams and 3 mg/kg/day for fetuses.

**Study title: Study on the development of myocardial injuries by S-4522 in rabbit fetuses**

**Key study findings:** S-4522 was administered to dams during (days 6-18, Exp. 1) and after (days 16-27, Exp. 2) the fetal organogenesis period at dose of 3 mg/kg/day. In dams, toxicity was observed in kidney, liver and gallbladder in dams treated at 3 mg/kg either during or after fetal organogenesis. However, no apparent cardiac toxicity was noted, that is different from the previous study where severe cardiac toxicity was observed at the same dosing levels. In fetus, toxicity was also noted in renal and liver in both experiments either during or after fetal organogenesis. No apparent cardiac or gallbladder toxicity was noted. Significant increased number of dead fetuses and decreased fetus body weight were observed in the fasted group receiving 3 mg/kg S-4522 as compared with those in the non-fasted and fasted control groups.

**Study no.:** A1-05-01

**Volume #, and page #:** electronic submission file name: a10501950425.pdf

**Conducting laboratory and location:** [redacted]

**Date of study initiation:** November 1994
**GLP compliance:** Yes
**QA reports:** yes ( ) no (X )
**Drug, lot #, radiolabel, and % purity**: lot number 56
**Formulation/vehicle:** water for injection

**Methods:**

Species/strain: rabbits, Kbl:JW
Doses employed: 0 and 3 mg/kg
Route of administration: oral gavage, 5 ml/kg
Study design: two experiments were conducted. The administration was given once daily between days 6 and 18 of pregnancy in Exp. 1 and between days 16 and 27 of pregnancy in Exp. 2.

Exp.1(EXP No.9023E1)

| Group | Dose (mg/kg/day) | Feed | Administration period | Autopsy date | No. of dams |
|---|---|---|---|---|---|
| A | Control | Ordinary | Day 6-18 | Day 19 | 4(4)*2 |
| B | 3 | Ordinary | Day 6-18 | Day 19 | 5(5) |
| C | Control | Fasting*1 | Day 6-18 | Day 19 | 3(4) |
| D | 3 | Fasting | Day 6-18 | Day 19 | 4(5) |

Exp.2(EXP No.9023E2)

| Group | Dose (mg/kg/day) | Feed | Administration period | Autopsy date | No. of dams |
|---|---|---|---|---|---|
| A | Control | Ordinary | Day 16-27 | Day 28 | 4(4)*2 |
| B | 3 | Ordinary | Day 16-27 | Day 28 | 5(5) |

*1:Fasted from day 12 to day 19 of pregnancy
*2:Number of copulated rabbits in parenthesis

Number/sex/group: 3-5 dams
Parameters and endpoints evaluated: Clinical observations, bodyweight and food consumption were recorded for all parental females. On days 19 and 28 of pregnancy in Exp. 1 and Exp. 2, histopathologic evaluation were performed in heart, liver, kidney and gallbladder for both dams and fetus.

**Results:**

Mortality: None.

Clinical signs: no treatment related change.

Body weight: no apparent treatment related changes.

Food consumption: in Exp. 1, food consumption decreased 32% on day 19 in the non-fasted treated group. No apparent change was noted in fasted group. In Exp. 2, no apparent change was noted.

Toxicokinetics: no data available.

Terminal and necroscopic evaluations:

**Dams:**

**Gross pathology:** in Exp. 1, one dam (D204) in the fasted S-4522 group showed adhesion of a white material on the epicardium and numerous fine cyst formation in the renal capsule.

Effect of S-4522 on fetal myocardium in rabbits(1) (A1-05-01)

Gross findings in FD pregnant rabbits -Summary- Female

| Organ Findings | Group | -- A --- Control | -- B --- S-4522 3 mg/kg | -- C --- Control | -- D --- S-4522 3 mg/kg |
|---|---|---|---|---|---|
| | Compound Dose | | | | |
| | Presence | yes no | yes no | yes no | yes no |
| Heart | | | | | |
| (No. affected / No. examined) | | ( 0/ 4) | ( 0/ 5) | ( 0/ 3) | ( 1/ 4) |
| epicardium,white material adhesion | | 0 4 | 0 5 | 0 3 | 1 3 |
| Kidney | | | | | |
| (No. affected / No. examined) | | ( 0/ 4) | ( 0/ 5) | ( 0/ 3) | ( 1/ 4) |
| bilateral,subcapsule,cyst | | 0 4 | 0 5 | 0 3 | 1 3 |
| Liver | | | | | |
| (No. affected / No. examined) | | ( 0/ 4) | ( 0/ 5) | ( 0/ 3) | ( 0/ 4) |
| Gallbladder | | | | | |
| (No. affected / No. examined) | | ( 0/ 4) | ( 0/ 5) | ( 0/ 3) | ( 0/ 4) |

*,** Statistically significant against group A at P<0.05 and P<0.01

In Exp. 2, one dam (A201) in the control group showed a black point (2 mm in diameter) at the apex of the right cardiac ventricle. In the another dam (A203), fading of the kidneys were observed, with opacity in the cortico-medullary junction in the right kidney and fine red points in the left renal cortex. In the treated group, one dam (B201) showed a yellow change of the kidney, and another dam (B202) showed fading of the heart, a yellow change of the liver and small gallbladder.

Effect of S-4522 on fetal myocardium in rabbits(2) (A1-05-01)

Gross findings in F0 pregnant rabbits -Summary- Female

| Organ | Findings | Group | A | B |
|---|---|---|---|---|
| | | Compound | Control | S-4522 |
| | | Dose | | 3 mg/kg |
| | | Presence | yes no | yes no |
| Heart | | | | |
| | (No. affected / No. examined) | | ( 1/ 4) | ( 1/ 5) |
| | fading | | 0 4 | 1 4 |
| | right ventricular,apex,black | | 1 3 | 0 5 |
| Kidney | | | | |
| | (No. affected / No. examined) | | ( 1/ 4) | ( 1/ 5) |
| | yellow | | 0 4 | 1 4 |
| | left,cortex,red point | | 1 3 | 0 5 |
| | right,cortico-medullary junction,opacity | | 1 3 | 0 5 |
| | bilateral,fading | | 1 3 | 0 5 |
| Liver | | | | |
| | (No. affected / No. examined) | | ( 0/ 4) | ( 1/ 5) |
| | yellow | | 0 4 | 1 4 |
| Gallbladder | | | | |
| | (No. affected / No. examined) | | ( 0/ 4) | ( 1/ 5) |
| | small | | 0 4 | 1 4 |

*,** Statistically significant against group A at P<0.05 and P<0.01

**Organ weight**: In Exp. 1, the kidney weights were significantly increased in the fasted S-4522 group. The liver weights in the fasted control group were significant lower than that in the non-fasted control group. The liver weight in the fasted S-4522 group was comparable to that in the non-fasted S-4522 group.

Effect of S-4522 on fetal myocardium in rabbits(1) (A1-05-01)

Absolute organ weights in F0 pregnant rabbits << Day 19 >>

| Group | Fin.BW kg | Heart g | Kidney g | Liver g |
|---|---|---|---|---|
| A: Control | | | | |
| Mean | 3.32 | 7.29 | 15.20 | 92.9 |
| S.D. | 0.31 | 1.10 | 1.33 | 7.0 |
| N | 4 | 4 | 4 | 4 |
| B: S-4522 3 mg/kg | | | | |
| Mean | 3.27 | 7.40 | 16.10 | 77.7 |
| S.D. | 0.21 | 0.49 | 1.43 | 17.6 |
| N | 5 | 5 | 5 | 5 |
| C: Control | | | | |
| Mean | 3.13 | 8.04 | 14.19 | 62.6** |
| S.D. | 0.04 | 0.54 | 2.54 | 4.8 |
| N | 3 | 3 | 3 | 3 |
| D: S-4522 3 mg/kg | | | | |
| Mean | 3.10 | 7.31 | 21.76* | 72.9* |
| S.D. | 0.16 | 0.39 | 5.24** | 9.5 |
| N | 4 | 4 | 4 | 4 |

*,** Statistically significant against group A at P<0.05 and P<0.01

$,$$ Statistically significant against group C at P<0.05 and P<0.01 (Expressed only group D)

**Histopathology:**

Exp. 1:

*Heart:* No abnormal changes were observed in any groups.

*Kidney:* Non-fasted control group: No abnormal changes were observed.

Non-fasted S-4522 group: Renal tubular injuries such as tubular necrosis, regeneration, dilatation and/or intraluminal cell debris were observed in 4/5 dams.

Fasted control group: No abnormal changes were observed.

Fasted S-4522 group: Renal tubular injuries mainly consisting of tubular necrosis, regeneration, dilatation, intraluminal cell debris and/or hyaline casts were observed in all dams (4/4). These changes were apparently more intense than those in the Non-fasted S-4522 group.

*Liver:* Non-fasted control group: No abnormal changes were observed.

Non-fasted S-4522 group: Fatty degeneration in the perilobular hepatocytes, single cell necrosis of the hepatocytes and activation of the Kupffer cells were observed.

Fasted control group: Diffuse fatty degeneration of the hepatocytes involving the entire hepatic lobules, atrophy of the hepatocytes in the centrilobular area and dilatation of the sinusoidal capillaries and irregular arrangement of the liver cell cords were observed.

Fasted S-4522 group: Fatty degeneration of the hepatocytes was observed in the mid-zonal to perilobular areas in all dams (4/4). Hepatocellular injuries/degeneration such as single cell necrosis, cytoplasmic inclusion bodies and/or ballooning or irregular arrangement of the liver cell cords and/or activation of the Kupffer cells were observed in 3/4 dams. These hepatocellular injuries and degenerative changes were apparently more intense in the fasted S-4522 group as compared with those in other groups.

*Gallbladder:* Non-fasted control group: No abnormal changes were observed.

Non-fasted S-4522 group: Vacuolation of the mucosal epithelium, mucosal erosion and inflammatory cell infiltration in the lamina propria mucosae were observed in one dam, and mucosal edema in another dam.

Fasted control group: Mucosal erosion and inflammatory cell infiltration in the lamina propria mucosae were observed.

Fasted S-4522 group: The mucosa showed epithelial changes such as vacuolation, single cell necrosis and increased cell division, edema or inflammatory cell infiltration in the lamina propria mucosae and erosion. The gallbladder mucosa was affected in all dams. The vacuoles in the mucosal epithelial cells were identified as the accumulation of the PAS-positive materials. These changes were clearly more intense and more extensive than those in the non-fasted S-4522 group.

Microscopic findings in FO pregnant rabbits

| Organ | Findings | Group A: Control | Group B: S-4522 3 mg/kg | | |
|---|---|---|---|---|---|
| | | Animal No. | AAAA 2222 0000 1234 | BBBBB 22222 00000 12345 | CCC DDDD 222 2222 000 0000 124 1245 |
| | | AKB | | | |
| | | Day | 1111 9999 | 11111 99999 | 111 1111 999 9999 |
| | | Fate | KKKK SSSS | KKKKK SSSSS | KKK KKKK SSS SSSS |
| Heart | | | ---- | ----- | --- ---- |
| Kidney | | | ---A | AA-AA | --- AAAA |
| | cortico-medullary junction,mineralization | | 1 | | |
| | cortico-medullary junction,outer medulla,tubular necrosis | | 2 | 13 | 3 |
| | cortico-medullary junction,outer medulla,tubular mineralization | | | 1 | |
| | cortico-medullary junction,outer medulla,tubular regeneration | | 13 | 12 | 3 3 |
| | cortico-medullary junction,outer medulla,tubular dilatation | | 1 | 11 | 2 1 |
| | cortico-medullary junction,outer medulla,cortex, tubular necrosis | | 3 | | 3 |
| | tubular lumen,cell debris | | | 2 | 2 2 |
| | cortex,cyst | | | | P |
| | medulla,cortex,tubular necrosis | | | | 4 3 |
| | medulla,cortex,hyaline cast | | | | 3 32 |
| | medulla,cortex,tubular regeneration | | | | 4 3 |
| | medulla,cortex,tubular dilatation | | | | 3 |
| | interstitium,inflammatory cell infiltration | | 11 | | 22 |
| | interstitium,inflammatory cell infiltration, perivascular | | | | 3 |
| Liver | | | ---- | AA-A- | AAA AAAA |
| | hepatic cell cord,irregularity | | 3 | 1 | 2 3 32 |
| | hepatocyte,ballooning | | | | 3 |
| | hepatocyte,inclusion body | | | | 2 |
| | hepatocyte,fatty degeneration,diffuse | | | 444 | |
| | hepatocyte,single cell necrosis | | 1 | | 33 |
| | Kupffer cell,activation | | 1 | | 3 33 |
| | sinusoid,dilatation | | | 222 | |
| | centrilobular zone,hepatocyte,atrophy | | | 321 | 2 |
| | perilobular zone,hepatocyte,fatty degeneration | | 33 | 1 | 3323 |
| Gallbladder | | | ---- | -A-A- | AA- AAAA |
| | mucosa,erosion | | | P | P P |
| | lamina propria,edema | | | 2 | 1 |
| | lamina propria,inflammatory cell infiltration | | 2 | | 23 1 22 |
| | epithelium,mitosis | | | | 2 |
| | epithelium,vacuolization | | 2 | | 133 |
| | epithelium,single cell necrosis | | | | 1 |
| | content,mineralization | | | P | |

KS: Scheduled kill
-:Normal finding A:Abnormal finding
Arbitrary grade; P:presence 1:very slight 2:slight 3:moderate 4:severe

**Exp. 2:**

*Heart:* No abnormal changes were observed either in the control or treated groups.

*Kidney:* Control group: Tubular changes such as regeneration, degeneration, dilatation and mineralization and interstitial fibrosis were observed.

S-4522 group: The renal tubules showed necrosis, regeneration, dilatation and/or mineralization.

*Liver:* Control group: Diffuse fatty degeneration, irregular arrangement of the liver cell cords, dilatation of the sinusoidal capillaries and centrilobular atrophy of the hepatocytes were observed.

S-4522 group: Irregular arrangement of the liver cell cords, balloon-like swelling or single cell necrosis and/or cytoplasmic inclusion bodies of the hepatocytes and activation of the Kupffer cells and inflammatory cell infiltration were observed in the mid-zonal to perilobular areas. Fatty degeneration of the hepatocytes was also observed, and this change was diffusely distributed over the entire lobules in some but was distributed mainly in the perilobular area in all animals including these two animals.

*Gallbladder*: Control group: The gallbladder mucosa showed epithelial vacuolation and inflammatory cell infiltration in the lamina propria mucosae.

S-4522 group: The gallbladder mucosa showed epithelial vacuolation and inflammatory cell infiltration in the lamina propria mucosae. The increased cell division of the mucosal epithelial cells was also observed.

Microscopic findings in FO pregnant rabbits

Group A: Control  Group B: S-4522 3 mg/kg

| Organ | Findings | Animal No. | AAAA BBBBB 2222 22222 0000 00000 1234 12345 |
|---|---|---|---|
| | | AKB | |
| | | Day | 2222 22222 8888 88888 |
| | | Fate | KKKK KKKKK SSSS SSSSS |
| Heart | | | ---- ----- |
| Kidney | | | --A- -A-AA |
| | cortico-medullary junction,tubular mineralization | | 1 |
| | cortico-medullary junction,outer medulla,tubular mineralization | | 1 |
| | cortico-medullary junction,outer medulla,tubular regeneration | | 2 2 |
| | cortico-medullary junction,outer medulla,tubular dilatation | | 1 2 |
| | cortico-medullary junction,outer medulla,cortex, tubular necrosis | | 3 33 |
| | cortico-medullary junction,outer medulla,cortex, tubular regeneration | | 2 |
| | medulla,cortex,tubular degeneration | | 2 |
| | medulla,cortex,hyaline cast | | 1 |
| | medulla,cortex,tubular regeneration | | 3 |
| | medulla,cortex,tubular dilatation | | 3 |
| | interstitium,fibrosis | | 1 |
| Liver | | | ---A AA-AA |
| | inflammatory cell infiltration | | 2 |
| | hepatic cell cord,irregularity | | 2 13 2 |
| | hepatocyte,ballooning | | 1 |
| | hepatocyte,inclusion body | | 1 1 |
| | hepatocyte,fatty degeneration,diffuse | | 4 33 |
| | hepatocyte,single cell necrosis | | 2 2 |
| | Kupffer cell,activation | | 2 |
| | sinusoid,dilatation | | 2 |
| | centrilobular zone,hepatocyte,atrophy | | 3 |
| | perilobular zone,hepatocyte,fatty degeneration | | 32 |
| Gallbladder | | | ---A ---AA |
| | lamina propria,inflammatory cell infiltration | | 1 12 |
| | epithelium,mitosis | | 1 |
| | epithelium,vacuolization | | 2 12 |

KS: Scheduled kill
-:Normal finding A:Abnormal finding
Arbitrary grade: 1:very slight 2:slight 3:moderate 4:severe

**Offspring:**

*Viability, body weight and external examination of fetuses:*

Exp. 1:

The numbers of corpora lutea of pregnancy and implantations were not significantly different among any groups. The number of live fetuses increased significantly in the non-fasted S-4522 group. In the fasted S-4522 group, the number of live fetuses decreased significantly as compared with that in the fasted control group. The number of dead fetuses increased significantly in the fasted S-4522 group as compared with those in the non-fasted and fasted control groups. When these dead fetuses in the fasted S-4522 group are classified, the number of resorbed fetuses increased significantly as compared with that in the non-fasted and fasted control groups. The fetal viability index was 94% in the non-fasted control group, 96% in the non-fasted S-4522 group and 96% in the fasted control group, whereas it was 58% in the fasted S-4522 group. The fetal body weight was 2.24 g in the non-fasted control group, 2.19 g in the non-fasted S-4522 group and 2.28 g in the fasted control group, whereas it was 2.02 g in the fasted S-4522 group, revealing a decreasing tendency but without any significant difference. In the external examination, no external anomaly was observed in any fetuses.

Effect of S-4522 on fetal myocardium in rabbits(1) (A1-05-01)

Inspection of F1 fetal development (Litter unit) -Summary-

| Group | No. of dams | No. of c.lutea | No. of implants | Implant ratio(%) | Live fetuses Tot. | Dead fetuses Tot. | Res. | Mac. | Dead | Abo. | Fetal viab(%) | Postimpl loss(%) | Body weight(g) Tot. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A: Control | | | | | | | | | | | | | |
| Mean | | 9.5 | 8.0 | 88 | 7.5 | 0.5 | 0.5 | 0.0 | 0.0 | 0.0 | 94 | 6 | 2.24 |
| S.D. | | 1.9 | 1.6 | 25 | 1.9 | 1.0 | 1.0 | 0.0 | 0.0 | 0.0 | 13 | 13 | 0.14 |
| N | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| B: S-4522 3 mg/kg | | | | | | | | | | | | | |
| Mean | | 11.0 | 10.8 | 98 | 10.4* | 0.4 | 0.4 | 0.0 | 0.0 | 0.0 | 96 | 4 | 2.19 |
| S.D. | | 0.7 | 0.8 | 4 | 1.1 | 0.5 | 0.5 | 0.0 | 0.0 | 0.0 | 5 | 5 | 0.31 |
| N | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| C: Control | | | | | | | | | | | | | |
| Mean | | 11.3 | 10.3 | 91 | 10.0 | 0.3 | 0.3 | 0.0 | 0.0 | 0.0 | 96 | 4 | 2.28 |
| S.D. | | 1.5 | 2.3 | 9 | 2.6 | 0.6 | 0.6 | 0.0 | 0.0 | 0.0 | 6 | 6 | 0.13 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| D: S-4522 3 mg/kg | | | | | | | | | | | | | |
| Mean | | 10.8 | 9.3 | 86 | 5.3$_{\#\#}$ | 4.0$^{**}_{\#\#}$ | 2.8$^{**}_{\#}$ | 1.3 | 0.0 | 0.0 | 58 | 42 | 2.02 |
| S.D. | | 1.3 | 1.5 | 9 | 1.0 | 1.6$_{\#\#}$ | 1.5$_{\#}$ | 1.5 | 0.0 | 0.0 | 13 | 13 | 0.25 |
| N | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

Implant ratio (%) = (No. of implants / No. of corpora lutea) X 100
Fetal viab (%) = (No. of live fetuses / No. of implants) X 100
Postimpl loss (%) [Post implant loss(%)] = ((No. of implants - No. of live fetuses) / No. of implants) X 100
c.lutea : Corpora lutea
*,** Statistically significant against group A at P<0.05 and P<0.01

#,## Statistically significant against group C at P<0.05 and P<0.01 (Expressed only group D)

**Exp. 2:**

The numbers of corpora lutea of pregnancy and implantations were not significantly different between the control and treated groups. The implantation index was 95% in the control group and 67% in the S-4522 group. However, it did not considered drug-related. The fetal viability index, fetal body weight and sex ratio were not significantly different between the treated and control groups. In the external examination, no external anomaly was observed in any fetuses.

Effect of S-4522 on fetal myocardium in rabbits(2) (A1-05-01)

Inspection of F1 fetal development (Litter unit) -Summary-

| Group | No. of dams | No. of c.lutea | No. of implants | Implant ratio(%) | Live fetuses Tot. | Male | Fem. | Dead fetuses Tot. | Res. | Mac. | Dead | Abo. | Fetal viab(%) | Postimpl loss(%) | Body weight(g) Tot. | Male | Fem. | Sex ratio (male/female) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A: Control | | | | | | | | | | | | | | | | | | |
| Mean | | 9.3 | 8.8 | 95 | 8.5 | 4.8 | 3.8 | 0.3 | 0.3 | 0.0 | 0.0 | 0.0 | 97 | 3 | 31.82 | 32.92 | 30.58 | 1.27 |
| S.D. | | 1.0 | 0.5 | 6 | 0.6 | 0.5 | 1.0 | 0.5 | 0.5 | 0.0 | 0.0 | 0.0 | 6 | 6 | 7.06 | 6.45 | 7.37 | ( 19/ 15) |
| N | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | |
| B: S-4522 3 mg/kg | | | | | | | | | | | | | | | | | | |
| Mean | | 9.0 | 6.2 | 67 | 5.2 | 2.8 | 2.4 | 1.0 | 0.6 | 0.4 | 0.0 | 0.0 | 80 | 20 | 36.38 | 39.31 | 32.69 | 1.17 |
| S.D. | | 2.3 | 3.6 | 28 | 3.4 | 3.1 | 1.1 | 1.0 | 0.9 | 0.9 | 0.0 | 0.0 | 22 | 22 | 5.20 | 5.09 | 9.53 | ( 14/ 12) |
| N | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | |

Implant ratio (%) = (No. of implants / No. of corpora lutea) X 100
Sex ratio = Total No. of male fetuses / Total No. of female fetuses
Fetal viab (%) = (No. of live fetuses / No. of implants) X 100
Postimpl loss (%) [Post implant loss(%)] = ((No. of implants - No. of live fetuses) / No. of implants) X 100
c.lutea : Corpora lutea
*,** Statistically significant against group A at P<0.05 and P<0.01

*Histopathology of the fetal organs*

Exp. 1:

*Heart:* No abnormal changes were observed in any groups.

*Kidney:* Non-fasted control group: No abnormal changes were observed.

Non-fasted S-4522 group: The renal tubules were dilated.

Fasted control group: The renal tubules were dilated.

Fasted S-4522 group: No abnormal changes were observed.

*Liver:* Non-fasted control group: No abnormal changes were observed.

Non-fasted S-4522 group: Fatty infiltration and dilatation of the sinusoidal capillaries were observed. These changes were of a very slight degree.

Fasted control group: Fatty infiltration, hepatocellular atrophy and dilatation of the sinusoidal capillaries were observed. In one fetus, hepatocellular atrophy and dilatation of the sinusoidal capillaries were marked, and the liver was congested.

Fasted S-4522 group: Fatty infiltration, hepatocellular atrophy and dilatation of the sinusoidal capillaries were observed. Fatty infiltration was slightly marked in the fasted control group, mild in the fasted S-4522 group and slight in the non-fasted S-4522 group.

*Gallbladder*: No abnormal changes were observed in any groups.

Microscopic findings in rabbit fetuses of experiment 1 (A1-05-01)

| | Control: ordinary | | | | | | | | 3 mg/kg: ordinary | | | | | | | | | | Control: fasting | | | | | | 3 mg/kg: fasting | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal No. | A | A | A | A | A | A | A | A | B | B | B | B | B | B | B | B | B | B | C | C | C | C | C | C | D | D | D | D | D | D | D | D |
| | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 | 1 | 2 | 2 | 3 | 3 | 4 | 4 | 1 | 1 | 2 | 2 | 3 | 3 | 4 | 4 | 5 | 5 | 1 | 1 | 2 | 2 | 4 | 4 | 1 | 1 | 2 | 2 | 4 | 4 | 5 | 5 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Organ Findings | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Liver | - | - | - | - | - | - | - | - | - | - | A | A | - | - | - | A | A | - | A | A | A | A | A | A | A | A | A | A | - | - | A | A |
| congestion | | | | | | | | | | | | | | | | | | | | | | 2 | | | | | | | | | | |
| fatty infiltration | | | | | | | | | | | 1 | 2 | | | | | 1 | | 2 | 3 | 1 | | 3 | 2 | 1 | 2 | 1 | 2 | | | 2 | 2 |
| hepatocyte / atrophy | | | | | | | | | | | | | | | | | | | 1 | | 2 | 3 | 2 | 2 | 1 | 1 | | 2 | | | | 2 |
| sinusoid / dilatation | | | | | | | | | | | | 1 | | | | 1 | 1 | | | 1 | 1 | 4 | 2 | 1 | | 1 | | 1 | | | | 1 |
| Gallbladder | - | - | - | - | - | - | - | - | - | D | - | - | - | - | D | D | - | - | - | - | - | D | - | - | - | - | - | - | - | - | - | - |
| Kidney | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - | - |
| tubular dilatation | | | | | | | | | | | | 2 | | | | | 1 | | | | 1 | | | | | | | | | | | |
| Heart | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

-: Normal finding A: Abnormal finding D: Tissue absent on preparation
Arbitrary grade; 1: very slight 2: slight 3: moderate P: presence

**Exp. 2:**

*Heart:* No abnormal changes were observed either in the treated or control group.

*Kidney:* Control group: No abnormal changes were observed.

S-4522 group: Hypoplasia (in addition to the small size, the differentiation of the nephron was delayed, and the number of the nephron was scanty) was observed in one fetus.

*Liver:* Control group: No abnormal changes were observed.

S-4522 group: Congestion and increased extramedullary hematopoiesis were observed.

*Gallbladder*: No abnormal changes were observed either in the treated or control group.

Microscopic findings in rabbit fetuses of experiment 2 (A1-05-01)

| | Control: ordinary | | | | | | | | 3 mg/kg: ordinary | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal No. | A 2 0 1 0 | A 2 0 1 0 | A 2 0 2 0 | A 2 0 2 0 | A 2 0 3 0 | A 2 0 3 0 | A 2 0 4 0 | A 2 0 4 0 | B 2 0 1 0 | B 2 0 1 0 | B 2 0 2 0 | B 2 0 2 0 | B 2 0 3 0 | B 2 0 3 0 | B 2 0 4 0 | B 2 0 4 0 | B 2 0 5 0 | B 2 0 5 0 |
| Organ / Findings | 1 | 4 | 1 | 3 | 2 | 3 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 3 | 1 | 2 | 2 | 3 |
| Liver | - | - | - | - | - | - | - | - | - | - | A 1 | A 2 | - | - | - | - | A 3 | A |
| congestion | | | | | | | | | | | | | | | | | 3 | |
| hyperplasia of extramedullary hematopoiesis | | | | | | | | | | | | | | | | | 3 | |
| Gallbladder | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Kidney | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - |
| dysgenesis | | | | | | | | | | | | | | | | | P | |
| Heart | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

-: Normal finding A : Abnormal finding

Arbitrary grade ; 1 : very slight 2 : slight 3 : moderate P : presence

**Summary of individual study findings:**

S-4522 was administered to dams during (days 6-18, Exp. 1) and after (days 16-27, Exp. 2) the fetal organogenesis period. In both experiments, the dams were autopsied one day after the final dose. In Exp. 1, fasted and non-fasted groups were set up, and, in Exp. 2, only normally fed groups were set up. The dose of S-4522 was 3 mg/kg/day.

**Exp. 1 (dosing during fetal organogenesis):**

1. No death nor moribund sacrifice occurred.
2. In the fasted S-4522 group, the viability and intrauterine growth of the fetuses tended to decrease, but no fetuses had any external anomalies.
3. No myocardial injuries were observed in dams. Renal, hepatic and gallbladder injuries were observed, and these changes were more pronounced in the fasted S-4522 group.

4. In fetuses, no abnormal change was observed in the heart and gallbladder. Liver and renal changes were observed, but were not above control incidence. However, significant increased number of dead fetuses and decreased fetus body weight were observed in the fasted group receiving 3 mg/kg S-4522 as compared with those in the non-fasted and fasted control groups.

**Exp. 2 (dosing after organogenesis):**
1. No death nor moribund sacrifice occurred.
2. The viability and intrauterine growth, and the external anomalies of the fetuses were not affected.
3. In the S-4522 group, no myocardial injuries were observed in any dams. The renal, hepatic and gallbladder injuries were observed in the treated dams.
4. In fetuses, no abnormal change was observed in the heart and gallbladder. Liver and renal changes were observed, but were not significantly increased above control.

Based on these results, dam toxicity was observed in kidney, liver and gallbladder in dams treated at 3 mg/kg during fetal organogenesis. However, no apparent cardiac toxicity was noted, that is different from the previous study where severe cardiac toxicity was observed at the same dosing levels. No apparent cardiac or gallbladder toxicity was noted in the fetuses. Renal and liver abnormalities were observed in the fetuses, but were not above control incidence. However, significant increased number of dead fetuses and decreased fetus body weight were observed in the fasted group receiving 3 mg/kg S-4522 as compared with those in the non-fasted and fasted control groups.

**Tissue distribution in the pregnant rat**

The placental transfer of radioactivity was investigated in pregnant rats following a single oral dose of [$^{14}$C]-rosuvastatin at 25 mg/kg on day 16 of pregnancy (Study: D4522 KMR033). At 30 minutes after dosing the concentrations of radioactivity in the fetal tissue and amniotic fluid were about 3% and 20%, respectively, of those in the maternal plasma (Individual plasma concentrations ranged between [redacted] ng equiv/g with a mean ± SE value of 792 ± 115 ng equiv/g). It was not determined which compounds were represented by the radioactivity in the fetus (eg, whether parent compound, metabolite or known impurity).

**D4522 KMR033 Individual and mean (± SE) levels of total radioactivity in selected organs, tissues and body fluids at 30 minutes post-dose following a single oral administration of [$^{14}$C]-ZD4522 to pregnant female rats at a target dose level of 25 mg/kg**

**Results expressed as ng equiv/g free acid**

| Tissue Type | Animal Number | | | | | | |
|---|---|---|---|---|---|---|---|
| | 001F | 002F | 003F | 004F | 005F | Mean | ± SE |
| Amniotic fluid | | | | | | 165 | 26.5 |
| Foetal tissue | | | | | | 20.8 | 2.67 |
| Kidneys (maternal) | | | | | | 969 | 380 |
| Liver (maternal) | | | | | | 40101 | 12000 |
| Mammary tissue | | | | | | 222 | 71.7 |
| Ovaries | | | | | | 873 | 539 |
| Foetal placenta | | | | | | 93.8 | 17.1 |
| Uterus | | | | | | 410 | 124 |

In an equivalent study with unlabelled rosuvastatin (Study: D4522 KMR049) the fetal tissue concentrations of rosuvastatin were 1.5% of the maternal plasma concentration. Rosuvastatin was measured by ——— in both matrices. These studies therefore indicate a low level of transfer of rosuvastatin and related substances into the fetus.

**Tissue distribution in the pregnant rabbit**

The placental transfer of radioactivity was investigated in pregnant rabbits following a single oral dose of [$^{14}$C]-rosuvastatin at 1 mg/kg on day 18 of pregnancy (Study: D4522 KMB032). At 30 minutes after dosing, radioactivity was measurable in the maternal plasma (Individual plasma concentrations of total radioactivity ranged between —— ng equiv/g with a mean ± SE value of 12.2 ± 2.94 ng equiv/g. Individual plasma concentrations of ZD4522 ranged between —— ng equiv/g with a mean ± SE value of 4.75 ± 1.42 ng equiv/g). Radioactivity was detected in fetal tissue in only 1/4 animals (3.97 ng/g) and in fetal liver in 2/4 (0.84 and 4.42 ng/g).

**D4522 KMB032 Individual and mean (± SE) levels of total radioactivity in selected organs, tissues and body fluids at 30 minutes post-dose following a single oral administration of [$^{14}$C]-ZD4522 to pregnant female rabbits at a target dose level of 1 mg/kg**

**Results expressed as ng equiv/g free acid**

| Tissue Type | Animal Number | | | | | |
|---|---|---|---|---|---|---|
| | 001F | 002F | 003F | 004F | Mean * | ± SE |
| Amniotic fluid | [redacted] | | | | 37.6 | 36.8 |
| Foetal tissue | | LOD | LOD | LOD | 0.99 | 0.99 |
| Foetal liver | | LOD | | LOD | 1.32 | 1.05 |
| Kidneys (maternal) | | | | | 221 | 47.3 |
| Liver (maternal) | | | | | 456 | 162 |
| Mammary tissue | | | | | 4.02 | 0.95 |
| Ovaries | | | | | 4.59 | 0.93 |
| Foetal placenta | | | | | 5.25 | 3.63 |
| Uterus | | | | [redacted] | 16.4 | 12.7 |
| Total foetus (liver + tissue) | | LOD | LOD | | 1.13* | 1.02 |

The limit of detection (LOD), taken as [redacted] dpm once background has been subtracted
(LOD values taken as 0.00 in mean calculations)
LOD = value below the limit of detection
* = Individual values given as LOD are taken as 0.00 for calculation of mean value

In an equivalent study, using unlabelled rosuvastatin (Study: D4522 KMB048), fetal tissue concentrations of rosuvastatin were below the limit of quantification of the assay ([redacted] ng/g tissue). Since an impurity was known to be present in the radioactive dose (Study: D4522 KMB032) and no transfer of parent compound was subsequently demonstrated, transfer of relevant rosuvastatin related compounds across the placenta of rabbit was not demonstrated.

**Secretion into breast milk in the rat**

[$^{14}$C]-rosuvastatin was given as a single dose of 38.4 mg/kg to female lactating rats thirteen days post-partum (Study: D4522 KMR052) and milk and plasma samples taken at various times post-dose. Total radioactivity was measured in plasma and milk, and rosuvastatin concentrations in milk. Rosuvastatin and metabolites were detected in milk, with rosuvastatin accounting for approximately 50% of total radioactivity. Levels of radioactivity in milk were between 1 and 3 times those in plasma at the same timepoint.

**D4522 KMR052. Individual concentrations of radioactivity and ZD4522 in milk and plasma following oral administration of [$^{14}$C]-ZD4522 to lactating rats**

| | | Concentration of radioactivity (ng equiv/g) | | Concentration of ZD4522 (ng/ml) | Ratio milk : plasma |
|---|---|---|---|---|---|
| Time (hours) | Rat | Plasma | Milk | Milk | (radioactive ratio) |
| 0.5 | 1 | 663 | 572 | 273 | 0.86 |
| | 2 | 576 | 492 | 336 | 0.85 |
| | 3 | 1183 | 661 | 320 | 0.56 |
| 2 | 4 | 199 | 607 | 389 | 3.05 |
| | 5 | 358 | 775 | 391 | 2.16 |
| | 6 | 370 | 570 | 216 | 1.54 |
| 4 | 1 | 402 | 811 | 240 | 2.02 |
| | 2 | 200 | 636 | 328 | 3.18 |
| | 3 | 236 | 949 | 255 | 4.02 |
| 6 | 4 | 138 | 398 | 143 | 2.88 |
| | 5 | 292 | 608 | 236 | 2.08 |
| | 6 | 262 | 450 | 198 | 1.72 |
| 8 | 1 | 337 | 1440 | 955 | 4.27 |
| | 2 | 289 | 427 | 213 | 1.48 |
| | 3 | 237 | 482 | 244 | 2.03 |
| 12 | 4 | 90 | 332 | 173 | 3.69 |
| | 5 | 145 | 171 | 118 | 1.18 |
| | 6 | 233 | 425 | 196 | 1.82 |
| 24 | 1 | 112 | 168 | * | 1.50 |
| | 2 | 122 | 109 | 53 | 0.89 |
| | 3 | 157 | 144 | 69 | 0.92 |
| | 4 | 82 | 110 | 43 | 1.34 |
| | 5 | 76 | 108 | 63 | 1.38 |
| | 6 | 189 | 191 | 95 | 1.01 |

* Not enough sample for analysis

**Reproductive and developmental toxicology summary:**

The reproductive toxicity of rosuvastatin has been assessed in rat fertility and pre-/post-natal developmental toxicity studies, and rat and rabbit teratology studies.

In the rat fertility study, groups of 24 male and 24 female rats received oral doses of 0, 5, 15 or 50 mg/kg/day rosuvastatin. Males were treated for 9 weeks prior to mating and throughout mating, and females for 2 weeks before mating and throughout mating until day 7 of pregnancy (Study: F-03-L). The top dose of 50 mg/kg/day caused maternal toxicity (reduced body weight and food consumption), however, there were no effects on reproductive parameters (estrus, cycle, male and female fertility, ovulation, implantation and maintenance of pregnancy), pup morphology or survival.

In the rat teratology study, groups of 35 to 36 female rats were administered rosuvastatin orally at doses of 0, 25, 50 or 100 mg/kg/day from days 7 to 17 of pregnancy (Study: F-11-L). The top dose level of 100 mg/kg caused a small transient decrease in food consumption and increased liver weight (which was also detected at 50 mg/kg/day). In fetus, treatment related increases in visceral malformation (thymic remnant in neck at ≥ 25 mg/kg), and skeletal variations (lumbar rib, asymmetry of sternebra in 2 fetuses at 100 mg/kg) were observed. No effects were noted on the viability of the offspring, functional/behavioral development, learning ability or reproductive function of the offspring.

In a dose-range setting study to a pre-/post-natal developmental toxicity study, ten time-mated female rats per group received by the oral route, doses of 0, 75, 100, 125 or 150 mg/kg/day rosuvastatin from day 7 of gestation to day 22 of lactation (Study: TWR3088). Severe toxicity was seen at dose levels ≥ 75 mg/kg/day. These doses resulted in dams being killed or found dead during or shortly after parturition. There were also histological findings of effects in the liver and stomach, together with reduced pups born alive or surviving. At these doses in the $F_1$ generation there were increased incidences of whole litter losses (either missing, found dead or terminated within 4 days of parturition). Pup sex distribution was unaffected. A top dose level of 50 mg/kg/day was used in a pre- and post-natal developmental toxicity study (Study: TWR2899). In this study, mated female Sprague Dawley rats were dosed once daily, by oral gavage, with ZD4522 at dose levels of 0, 0, 2, 10 or 50 mg/kg/day from day 7 of gestation, throughout pregnancy and until day 21 of lactation. No apparent treatment related changes were observed either in the dams or the offspring at dose levels of up to 10 mg/kg/day. Lower number of pups live born and incidence of pups with eyes open on day 16 post partum, and increase in startle amplitude for males were noted in the 50 mg/kg. Renal toxicity was observed in dams at 50 mg/kg. Therefore, together with the previous study, the NOAEL was estimated to be 25 mg/kg/day for dams in the aspect of general toxicology and 10 mg/kg/day for maternal reproductive function, fetuses and offspring.

In a preliminary to a rabbit teratology study, groups of 3 female rabbits were dosed orally with 0, 1.25, 2.5, 5 or 10 mg/kg/day rosuvastatin for 14 days (Study: B-38-L). By the end of the dosing period, animals dosed with 5 and 10 mg/kg/day were showing signs of severe toxicity (including death at the top dose level, body weight loss, hypoactivity and debility).

At necropsy, there was evidence of effects in the gall bladder, kidney and urinary bladder, with supporting biochemical evidence of change in these organs and muscle. The main rabbit teratology study was also preceded by a preliminary study (Study: B38.Y2-L) in which 3 to 5 pregnant female rabbits per group received oral doses of 0, 1, 2 or 4 mg/kg/day rosuvastatin from days 6 to 18 of pregnancy. The top dose of 4 mg/kg resulted in 2 deaths accompanied by reduced body weight and food consumption, and in these two rabbits there was evidence, at necropsy, of effects in the kidney, gall bladder and stomach. The main teratology study used groups of 14 to 16 female rabbits which were given rosuvastatin orally at doses of 0, 0.3, 1 or 3 mg/kg/day from days 6 to 18 of pregnancy. This study showed that rosuvastatin was not teratogenic in the rabbit at doses ≤3 mg/kg/day (Study: B-38-L). At 3 mg/kg/day, however, some dams died with marked effects in the kidney (necrosis and mineralization of the cortical tubular epithelial cells), liver (vacuolation of the centrilobular hepatocytes) and in particular, heart and intercostal muscle (both muscles showed necrosis and mineralization). Despite these effects in the dams, there were no effects on the pups.

When 3 mg/kg/day rosuvastatin (Study: A1-05-01 950425) was dosed to fed and fasted rabbits during the period of fetal organogenesis, the toxicity findings in the main teratology study were reproduced with aggravation of the effects in fasting dams. Toxicity was observed in kidney, liver and gallbladder in dams treated at 3 mg/kg either during or after fetal organogenesis. However, no apparent cardiac toxicity was noted. In fetus, toxicity was not remarkable in heart and gallbladder compared to control. However, significant increased number of dead fetuses and decreased fetus body weight were observed in the fasted group treated with 3 mg/kg rosuvastatin as compared with those in the non-fasted and fasted control groups.

Rabbits appeared to be more sensitive to rosuvastatin than most other species, probably due to its higher exposure level; lower hepatic HMG-CoA reductase activity; and slow clearance. The $C_{max}$ at a single dose of 10 mg/kg in rabbits was 380 ng/ml. The $C_{max}$ at single dose of 5 mg/kg in rats or dogs was 42 or 92 ng/ml, respectively. The hepatic HMG-CoA reductase activity in rabbits were 1/10 to 1/200-fold lower than those in rats, mice, monkeys and dogs. Rabbits did not eliminate the drug as rapidly as other species, since it takes about 72 hours to excrete all the radioactive dose compared to others which are within 24 hours. More of the drug was excreted in the urine (19%) in rabbit compared to 1% in other species where predominately by fecal excretion. That could lead to the difference in toxicity, where rabbits had more renal toxicity and other species had more liver toxicity.

**Reproductive and developmental toxicology conclusions:**

Rosuvastatin induced fetal toxicity in rats at 25 mg/kg and rabbits at 3 mg/kg. In rats, both maternal toxicity (reduced body weight and food consumption, liver and renal toxicity) and fetal toxicity (lower number of pups live born, slight low fetal body weight, low incidence of pups with eyes open, and increase in startle amplitude, increases in visceral malformation and skeletal variations, and slightly retarded ossification) were observed at ≥ 25 mg/kg with NOAEL for dams and fetus of 15 mg/kg. In rabbits, severe maternal toxicity (mortality, body weight loss, hypoactivity and debility, and marked histopathologic changes in liver, gallbladder, kidney, heart, and muscle) and fetal toxicity (increase in dead fetuses, decrease in fetal viability index) were observed at 3 mg/kg with NOAEL for dams and fetus of 1 mg/kg. The corresponding exposure levels for rats at 25 mg/kg were 3, 6, 13, and 28X human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively. The AUC for pregnant rabbits at 3 mg/kg were not provided. Estimates based on the exposure ($C_{max}$) in male rabbits at dose of 5 mg/kg, the exposure for female rabbits at 3 mg/kg might be about ½, 1, 3, and 5X human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively.

There was a low distribution of rosuvastatin to fetus in rats (3% or 20% of maternal plasma concentration in fetal tissue or amniotic fluid, respectively) following a single oral dose of 25 mg/kg. Relative higher distribution in fetal tissue (25% maternal plasma concentration) was observed in 1/4 fetus in rabbits following a single oral dose of 1 mg/kg. However, in the lactating rat, rosuvastatin was found in milk at concentrations up to 3 times those in plasma. Theses data suggested that rosuvastatin have risk to pregnant women and nursing mothers.

Since cholesterol and other products of cholesterol biosynthesis are essential components for fetal development (including synthesis of steroids and cell membranes), and HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly the synthesis of other biologically active substances derived from cholesterol, they may cause fetal harm when administered to pregnant women. Therefore, Crestor should not be administered to pregnant women or nursing mothers.

## VIII. SPECIAL TOXICOLOGY STUDIES:

**Study title: Gallbladder toxicity of HMG CoA reductase inhibitors in mice**
Full review is attached on Page 66 of the initial pharmacology/toxicology review.
**Key study findings:** the gallbladder toxicities of S-4522 can be detected in mice at 250 and 500 mg/kg, and these toxicities are common to related compounds (simvastatin, lovastatin, and fluvastatin).

**Study title: Supplement toxicity studies of S-4522 in rats: toxicological characterization of effective compounds**
Full review is attached on Page 67 of the initial pharmacology/toxicology review.
**Key study findings:** the differences in the repeated-dose toxicities of S-4522 in rats is due to the differences in the dietary Ca content. The sensitivity of the rats to the S-4522 toxicities increases with decreasing Ca content in the diet.

**Study title: Supplement 3-month repeated oral dose toxicity study of S-4522 in dogs (examination of effects on gallbladder)**
Full review is attached on Page 69 of the initial pharmacology/toxicology review.
**Key study findings:** dogs were doses at 1, 2, and 4 mg/kg for 91 days. No apparent toxicity was noted. NOAEL was assumed to be 4 mg/kg/day.

**Study title: Preventive effect of mevalonate or farnesol on the toxicity induced by S-4522 in rats**
Full review is attached on Page 71 of the initial pharmacology/toxicology review.
**Key study findings:** In the group receiving 250 mg/kg S-4522 alone, all animals died or were killed moribund by day 14 of treatment. These animals showed marked elevations in blood GOT and GPT and nephrotoxicities in addition to the above changes. In the MV-supplemented groups, rats were given mevalonate at doses of 50 and 22 mg/kg. All toxicity findings were clearly ameliorated or prevented, and this protective effect was dose-dependent. In the FN-supplemented groups, rats were given Farnesol at doses of 50 and 200 mg/kg. The appearance of toxic changes was delayed, and the survival time was prolonged. However, the changes were similar to those seen in the S-4522 alone group. Based on these, S-4522-induced various changes are thought to be the consequence of the potent activity of the compound in inhibiting HMG CoA reductase.

**Study title: Supplement toxicity study of S-4522 in rabbits**
Full review is attached on Page 73 of the initial pharmacology/toxicology review.
**Key study findings:** S-4522 (5 and 10 mg/kg), Simvastatin (20 and 40 mg/kg) and Fluvastatin (10 and 20 mg/kg) induced significant toxicities in heart, skeletal muscle, liver, kidney. The toxicities of S-4522 were completely prevented by combining mevalonic acid (40 mg/kg), and the onset of these toxicities was correlated to the blood farnesyl pyrophosphate level. The toxicities of S-4522 were therefore thought to be directly linked to its inhibitory activity on HMG-CoA reductase.

**Study title: Effects of mevalonic acid on toxicities of S-4522 in dogs**

Full review is attached on Page 76 of the initial pharmacology/toxicology review.

**Key study findings:** gallbladder toxicities of 50 mg/kg S-4522 were ameliorated by coadministration of mevalonate (10 and 50 mg/kg), suggesting the toxicities of S-4522 were directly linked to its inhibitory activity on HMG-CoA reductase.

**Study title: Antigenicity and sensitisation studies**

**Key study findings:** The antigenic and sensitization potential of rosuvastatin have been evaluated in a series of studies. In a passive anaphylactic test using the mouse/rat model, serum collected from mice given repeated doses of rosuvastatin, either orally or by intraperitoneal injection over a 2 week period was used undiluted to inoculate and then challenge groups of 10 rats 24 hours after inoculation. The antigenic potential of rosuvastatin was also tested in a guinea pig model. Groups of 10 female guinea pigs received repeated doses of 50 or 500 mg/day either orally or by subcutaneous injection over a 2 week period (induction) and then were challenged intravenously with rosuvastatin at 4 and 24 hours, and after 8 days. In a maximization test using guinea pigs, a group of 10 female guinea pigs received 500 mg rosuvastatin by intradermal injection and then topical application on day 8 (induction) and then were challenged topically on day 21 with 100 mg or 1 mg rosuvastatin. All studies resulted in negative responses, suggesting rosuvastatin has no antigenic and sensitization potential.

The following toxicology studies were submitted in this NDA:

| Type of study/ Study number | Report Title | Species (Strain) | Duration | Route of administration | Dose (mg/kg/day) | Conform to GLP |
|---|---|---|---|---|---|---|
| **Acute toxicology** | | | | | | |
| T-Mizushima Y-001 (910624) | Preliminary Toxicity Study of 45-2204 in Rats | Rat (F344) | | Oral<br>Intravenous | 1000 and 2000<br>250 and 500 | No |
| S-4522-B-15-L | Single Oral Dose Toxicity Study of S-4522 in Rats | Rat (SD) | | Oral | 1000 and 2000 | Yes (MHW)[*1] |
| S-4522-B-16-L | Single Oral Dose Toxicity Study of S-4522 in Dogs | Dog (beagle) | | Oral | 1000 and 2000 | Yes (MHW) |
| **Repeat dose toxicology** | | | | | | |
| SGI 143/943218 (F-015.Y1L) | Toxicity Study in Mice by Repeated Oral Administration for 2 Weeks | Mouse (B6C3F1) | 2 weeks | Oral | 0, 20, 60 and 200 | Yes (UK) |
| SGI 144/952323 (F-15-L) | Toxicity Study to Mice by Repeated Oral Administration for 13 Weeks | Mouse (B6C3F1) | 13 weeks | Oral | 0, 20, 60 and 200 | Yes (UK) |
| T-Mizushima Y-001 (910910) | Preliminary Repeated Intravenous Dose Toxicity Study of 45-2204 and Pravastatin in Rats | Rat (SD, females) | 5 days | Intravenous | 200, 400 | No |
| T-Mizushima Y-001 (911125) | Preliminary Toxicity Study of S-4522: 14-Days Repeated Intravenous Dose Toxicity in Rats | Rat (SD, males) | 14 days | Intravenous | 0, 50, 100, 200 | No |
| T-Mizushima Y-001 (911004) | Preliminary Toxicity Study of 45-2204: 14-Days Repeated Oral Dose Toxicity in Rats | Rat (SD) | 14 days | Oral | 0, 125, 500 and 2000 nb $Na^+$-salt | No |
| T-Mizushima Y-001 (920217) | Preliminary Repeated Oral Dose Toxicity Study of S-4522 in Rats | Rat (SD; males only) | 14 days | Oral | 0, 125, 500, 2000 | No |
| S-4522-B-17-L | One-Month Repeated Oral Dose Toxicity of S-4522 in Rats | Rat (SD) | 1 month | Oral | 0, 15, 50, 150 | Yes (MHW) |
| S-4522-F-09-L | Three-Month Repeated Oral Dose Toxicity Study of S-4522 in Rats | Rat (SD) | 3 months | Oral | 0, 10, 30, 100 | Yes (MHW) |
| SGI 145/951544 (F-16-L) | Preliminary Carcinogenicity Study: Toxicity to Rats by Repeated Oral Administration for 13 Weeks | Rat (CDF F-344) | 13 weeks | Oral | 0, 6, 20, 60 | Yes (UK) |
| TKR3081 | 91-Day Oral (Gavage Administration) Toxicity Study in the Rat | Rat (SD) | 91 days | Oral | 0, 160 | Yes (UK) |
| S-4522-F-13-L | Six-Month Repeated Oral Dose Toxicity Study of S-4522 in Rats | Rat (SD) | 6 months | Oral | 0, 2, 6, 20 | Yes (MHW) |
| TAD1018 | 14-Day Intravenous Toxicity Study in Dogs | Dog (beagle) | 14 days | Intravenous | 0, 1, 5, 12 | Yes (UK) |
| T-Mizushima Y-001 (920529) | Pre-Toxicity Study of S-4522 in Beagle Dogs: 14-Day Repeated Oral Dose | Dog (beagle) | 14 days | Oral | 50, 200 | No |
| S-4522-B-20-L | One-Month Repeated Oral Dose Toxicity Study of S-4522 in Dogs | Dog (beagle) | 1 month | Oral | 0, 10, 30, 90 | Yes (MHW) |

| Type of study/ Study number | Report Title | Species (Strain) | Duration | Route of administration | Dose (mg/kg/day) | Conform to GLP |
|---|---|---|---|---|---|---|
| S-4522-B-21-L | Three-Month Repeated Oral Toxicity Study of S-4522 in Dogs | Dog (beagle) | 3 months | Oral | 0, 7.5, 15, 30 | Yes (MHW) |
| S-4522-B-37-L | Investigative 6-Month Repeated Oral Toxicity Study of S-4522 in Dogs | Dog (beagle) | 6 months | Oral | 1, 4 | Yes (MHW) |
| S-4522-B-46-L | Twelve-Month Repeated Oral Toxicity Study of S-4522 in Dogs | Dog (beagle) | 12 months | Oral | 0, 1, 3, 6 | Yes (MHW) |
| S-4522-F-10.B1-N | A Preliminary 30-Day Repeated Dose Toxicity Study of S-4522 Administered Orally to Cynomolgus Monkeys | Cynomolgus monkey | 30 days | Oral | 0, 10, 30 | No |
| S-4522-F-14-L | Six-Month Repeated Oral Toxicity Study of S-4522 in Monkeys | Cynomolgus monkey | 6 months | Oral | 0, 10, 30 | Yes (MHW) |
| **Genotoxicity** | | | | | | |
| S-4522-B-11-L | Reverse Mutation Test of S-4522 with Bacteria | Bacteria | | | | Yes (MHW) |
| TMV797 | L5178Y TK+/- Mouse Lymphoma Mutation Assay | Mouse lymphoma cells | | | | Yes (UK) |
| S-4522-B-25-L | Chromosomal Aberration Test of S-4522 in Cultured Chinese Hamster Cells | Cultured Chinese hamster cells | | | | Yes (MHW) |
| S-4522-B-24-L | Micronucleus Test of S-4522 with Mouse Bone Marrow Cells | Mouse (male) | 1 or 2 doses | Oral | 1 x 250-1000 or 2 x 125-500 | Yes (MHW) |
| TMV865 | Bacterial Mutation Assay in S.typhimurium and E.coli | Bacteria | | | | Yes (UK) |
| TMV899 | Bacterial Mutation Assay in S.typhimurium and E.coli | Bacteria | | | | Yes (UK) |
| TMV878 | Bacterial Mutation Assay in S.typhimurium and E.coli | Bacteria | | | | |
| TMV900 | Bacterial Mutation Assay in S.typhimurium and E.coli | Bacteria | | | | Yes (UK) |
| TYX111 | In Vitro Cytogenetic Study Using Cultured Human Lymphocytes | Cultured human lymphocytes | | | | Yes (UK) |
| TYX113 | In Vitro Cytogenetic Study Using Cultured Human Lymphocytes | Cultured human lymphocytes | | | | Yes (UK) |
| **Carcinogenicity** | | | | | | |
| TCM1088 | 107 Week Oral (Gavage Administration) Oncogenicity Study in the Mouse | Mouse (B6C3F1) | 104 weeks | Oral | 0, 10, 60, 200 (400) | Yes (UK) |
| TCR2852 | 104 Week Oral (Gavage Administration) Oncogenicity Study in the Rat | Rat (SD) | 104 weeks | Oral | 0, 2, 20, 60, 80 | Yes (UK) |

| Type of study/ Study number | Report Title | Species (Strain) | Duration | Route of administration | Dose (mg/kg/day) | Conform to GLP |
|---|---|---|---|---|---|---|
| **Reproduction studies** | | | | | | |
| S-4522-F-03-L (930520) | Study on Oral Administration of S-4522 Prior to and in the Early Stages of Pregnancy in Rats | Rat | 14 days | Oral | 0, 15, 50, 150, 450 | Yes (MHW) |
| S-4522-F-11-L | Study on Oral Administration during the Period of Organogenesis in Rats | Rat | 11 days | Oral | 0, 25, 50, 100 | Yes (MHW) |
| TWR3088 | Sighting Study for the Pre- and Post-Natal Study of Oral Administration ZD4522 in Rats | Rat | 45 days | Oral | 0, 75, 100, 125, 150 | Yes (UK) |
| TWR2899 | Pre-and Post-Natal Developmental Toxicity Study in Rats | Rat | 45 days | Oral | 0, 2, 10, 50 | Yes (UK) |
| S-4522-B-38-L | Study on Oral Administration of S-4522 during the Period of Fetal Organogenesis in Rabbits | Rabbit | 13 days | Oral | 0, 0.3, 1, 3 | Yes (MHW) |
| A1-05-01 (950425) | Effects of Drug on Organogenesis: Development of Myocardiopathy in Rabbit Fetuses with S-4522 | Rabbit | 12 or 13 days | Oral | 3 | No |
| **Special Toxicology studies** | | | | | | |
| **Tolerance studies** | | | | | | |
| TKN310 | In Vitro Blood Compatibility Study | n/a | n/a | n/a | n/a | Yes (UK) |
| **Antigenicity studies** | | | | | | |
| F-01-L | Antigenicity Study of S-4522 in Mice | mouse | | | | Yes (MHW) |
| F-02-L | Antigenicity Study of S-4522 in Guinea Pigs | guinea pig | | | | Yes (MHW) |
| B-28-L | Maximization Test of S-4522 | guinea pig | | | | Yes (MHW) |
| **Gall bladder** | | | | | | |
| A1-01-01 (931105) | Supplemental Toxicity Studies of S-4522 in Rats and Mice: Gallbladder Toxicity of HMG-CoA Reductase Inhibitors in Mice | Mouse | 14 days | Oral | 0, 250, 500 | No |
| S-4522-B-47-N | Study on the Gallbladder Toxicity of S-4522 in Mice: Comparison with Related Drugs | Mouse | 7 days | Oral | 0, 500 | No |
| S-4522-B-33-L | Supplement 3-Month Repeated Oral Dose Toxicity Study of S-4522 in Dogs (Examination of Effects on Gallbladder) | Dog | 3 months | Oral | 1, 2, 4 | Yes (MHW) |

| Type of study/ Study number | Report Title | Species (Strain) | Duration | Route of administration | Dose (mg/kg/day) | Conform to GLP |
|---|---|---|---|---|---|---|
| **Effect of mevalonate/farnesol** | | | | | | |
| S-4522-B-29-N-B (930831) | Evaluation of Blood Cholesterol-lowering Effect of S-4522 in Experimental Animals | Mouse | 7 days | Oral | 100 | No |
| S-4522-B-29-N-C (930128) | Evaluation of Blood Cholesterol-lowering Effect of S-4522 in Experimental Animals | Mouse | 7 days | Oral | 10, 100 | No |
| Y-Mizushima A1-01-01 (931022) | Supplemental Toxicity Studies of S-4522 in Rats and Mice: Protective Effect of Mevalonate on S-4522 Toxicity in Rats | Rat | 10 days | Oral | 0, 250 | No |
| T-Yahara I-001 (921202) (contains T-Yahara I-003 930112) | Preventive Effect of Mevalonate or Farnesol on Toxicity Induced by S-4522 in Rats | Rat | 14 days | Oral | 250 | No |
| S-4522-B-52-N | Supplement Toxicity Study of S-4522 in Rabbits | Rabbit | 14 days | Oral | 5, 10 | No |
| S-4522-B-34-N | Effects of Mevalonic Acid on the Toxicities of S-4522 in Dogs | Dog (beagle) | 30 days | Oral | 50 | No |
| **Effect of diet and husbandry** | | | | | | |
| A1-01-01 (940415) | Supplemental Toxicity Study of S-4522 in Rats and Mice: Differences of S-4522-Toxicity in Mice Fed with Diets CA-1 and MM-6 | Mouse (male) | 14 days | Oral | 0, 200, 300, 400 | No |
| T-Mizushima Y-001 (930217) | Supplement Toxicity Study of S-4522 in Rats: Influence of Food, Cage Bedding, and Rat Farm on S-4522 Repeated-Dose Toxicity in Rats | Rat (male) | 14 days | Oral | 150 | No |
| T-Mizushima A1-01-01 (950110) | Supplementary Toxicity Studies of S-4522 in Rat | Rat (male) | 8 days | Oral | 150 | No |
| T-Mizushima A1-01-01 (941031) | Supplemental Toxicity Study of S-4522 in Rat | Rat (male) | 8 days | Oral | 150 | No |
| I-Kato A1-01-01 (980617) | Supplemental Toxicity Study of S-4522 in Rats: Influence of Dietary Ca Content on S-4522 Toxicity | Rat (male) | 5 days | Oral | 150 | No |
| I-Kato A1-01-01 (980616) | Supplemental Toxicity Study of S-4522 in Rats: Influence of Feed on Plasma S-4522 Concentration | Rat (male) | 8 days | Oral | 150, 250 | No |
| T-Mizushima Y-001 (930712) | Supplement Toxicity Study of S-4522 in Rats: Influence of Food on Toxicity of HMG-CoA Reductase Inhibitors in Rats | Rat (male) | 8 days | Oral | 150 | No |
| T-Mizushima Y-001 (930719) | Supplement Toxicity Study of S-4522 in Rats: Studies of Hepatic Function with Indocyanine Green in Rats Fed CA-1 or MM-6 | Rat | | | | No |
| T-Yahara I-002 (921216) | Effect of S-4522 on Liver of Hamsters Fed an Atherogenic Diet Histopathological Findings | Hamster (male) | 14 days | Diet | 1 and 10 | No |
| **Other studies** | | | | | | |
| T-Mizushima Y-001 (930831) | Supplement Toxicity Study of S-4522 in Rats: Sex Difference in Repeated-Dose Toxicities of S-4522 and Simvastatin in Rats | Rat | 10 days | Oral | 250 | No |
| T-Mizushima Y-001 (920930) | Comparison of Toxicity Between Lots 52 and 54 of S-4522 | Rat (male) | 14 days | Oral | 150 | No |
| TAD1148 | One Month Oral Toxicity Study in Dogs | Dog (beagle) | | Oral | 0, 12 | Yes (UK) |

## IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

**Conclusions:** generally, studies were adequate to evaluate the safety of this compound and support the approval of this drug.

**General Toxicology Issues:**

Preclinical studies include toxicology studies in rats, dogs, mice and monkeys with duration of single dose to 12 months, 2-year carcinogenicity studies in mice and rats, genotoxic studies, reproductive toxicity studies in rats and rabbits, and special toxicology studies. Generally, the toxicology findings are similar to other approved statins. The major target organs were liver, gallbladder (dog, mouse), forestomach (rodents), cornea, lens and retina (dog), kidney, and muscle.

Liver is the major target of rosuvastatin in rats, mice, and dogs. The changes in liver include increases in plasma transaminases, hepatocyte hypertrophy, and cell necrosis. These findings are consistent with the selective distribution of rosuvastatin in liver. The Sponsor suggested that the liver toxicity to the structural/functional components be due to the depletion of cholesterol by prolonged and extensive inhibition of HMG-CoA reductase. Rats were the most sensitive species to the liver toxicity, probably due to its high rate of hepatic synthesis of cholesterol. Generally, liver toxicity was observed at exposure levels about 2, 1, and 7X the human exposure for mice, rats and dogs, respectively, based on the Sponsor proposed high dose of 80 mg/day; about 4, 2, and 16X the human exposure for mice, rats and dogs, respectively, based on the human dose of 40 mg/day; about 11, 5, and 35X the human exposure for mice, rats and dogs, respectively, based on the human dose of 20 mg/day; and about 24, 11, and 78X the human exposure for mice, rats and dogs, respectively, based on the human dose of 10 mg/day. Since liver toxicity was a known class effect for statins, it was closely monitored in clinical studies.

Toxicity on gallbladder and biliary duct, including lamina propia mucosa edema, hemorrhage and inflammatory infiltration, were observed in dogs. That was consistent with the excretion route of rosuvastatin. This toxicity was observed in dogs at 6 mg/kg with exposure levels 7, 16, 35, and 78X the human exposure at human doses of 80, 40, 20, and 10 mg/kg, respectively. Gallbladder toxicity was also observed in mice at 250 mg/kg ( >10X human exposure at human dose of 80 mg/day), but less severe than dogs. Gallbladder effects have also been observed with other drugs of this class.

Edema, hemorrhage and partial necrosis in the interstitium of the choroid plexus was observed in one female dog at 90 mg/kg ( 46X human exposure at human dose of 80 mg/day) that was sacrificed *in extremis* on day 24 of dosing. CNS lesions characterized by perivascular hemorrhage, edema, mononuclear cell infiltration, fibrinoid degeneration of vessel walls in the choroid plexus of the brain stem, and ciliary body of the eye have been observed with several drugs in this class. Toxicity on eyes including opacity of cornea and lens, and retina dysplasia was only observed in dogs at exposure levels 1/2, 1, 3 and 6X the human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively. Lenticular effects have been observed with other drugs of this class.

Forestomach toxicity (mucosal hyperkeratosis) was observed in rats at exposure levels 6, 12, 27, and 60X the human exposure at human doses of 80, 40, 20, and 10 mg/kg, respectively. This anatomical feature is unique to rodents and is therefore not considered clinically relevant.

Toxicity on endocrine organs were noted in testis (decrease in spermatogenic epithelium, giant cells and vacuolation in seminiferous tubular epithelium), pancreas (vacuolation of acinar cell), adrenal (necrosis of parenchyma) and thyroid (ectopic thymus) in monkeys at exposure levels 2, 4, 8, and 18X the human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively. Giant cells and/or mild tubular seminiferous degeneration were also observed in a one-month dog study at dose of 90 mg/kg. The effects on testis in dogs and monkeys have been seen with several drugs in this class.

Renal and muscle toxicity was seen in rats, dogs, rabbits, and monkeys. The toxicity was characterized by blood chemistry changes including increases in creatinine, CPK, urea nitrogen, and histopathologic changes including renal tubular cell degeneration /necrosis, and cardiac or intercostal muscle necrosis. Generally, renal and muscle toxicity was observed in animals dead or moribund killed after high level exposure to rosuvastatin with high multiples of human exposure (about 39 to 46X human exposure for rats and dogs, respectively, based on the human dose of 80 mg/day). The severity and low frequency nature of renal/muscle toxicity of rosuvastatin suggests that some individuals are more susceptible to rosuvastatin, presumably due to the great variations in individual exposure level in both humans and animals. Humans experiencing these type of adverse events had elevated drug plasma levels according to the medical reviewer. In addition, pre-existing condition of renal impairment will significantly enhance the risk of renal toxicity due to increased rosuvastatin plasma levels at such condition. Therefore, the potential risk of renal/muscle toxicity to human can not be excluded. Marked muscle toxicity was reported in humans when Lovastatin was combined with cyclosporin A, an immunosuppressant.