**Number/sex/dose:** 22/sex in control; 42/sex in treated. Only 10 animals/gp were used for toxicological evaluation. The remaining animals were used for toxicokinetics.
**Doses:** 4, 16, and 60 mg/kg/day
**Duration:** 13 weeks

Mortality: None treatment related.

Clinical signs: None related to treatment

Bodyweight: There was a non-significant decrease in BW gain in males and females.

| | Group and Dosage (mg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | male | | | | female | | | |
| | 0 | 4 | 16 | 60 | 0 | 4 | 16 | 60 |
| week 0-13 | 11.0 | 15.3 | 10.8 | 8.0 | 7.9 | 7.5 | 6.7 | 6.0 |
| SD | 2.74 | 5.05 | 2.04 | 1.99 | 2.93 | 2.36 | 2.23 | 2.38 |

Food Consumption; No significance compared to controls.

Clinical chem/hematology: Some early morning elevations of T3 for HD males and females and elevations of T4 for HD males.

Organ weights: Slight increase in relative liver and pituitary weight for HD males. Mean thymus wts were decrease in HD females.

Gross pathology: No treatment related effects.

Histopathology: No changes correlated with the organ wt changes except for a minimal centrilobular hepatocyte enlargement in 1/10 LD, 1/10 MD and 3/10 HD male mice.

**Sub-Acute Toxicity to Rats by Dietary Administration for 13 Weeks.**

**Testing facility:**
**Study number:** 9610716
**Date:** 10/96
**Species/strain:** CD Sprague-Dawley BR Rats
**Route:** oral; mixed with diet
**Number/sex/dose:** 18/sex in control; 26/sex in treated. Only 10 animals/gp were used for toxicological evaluation. The remaining animals were used for toxicokinetics.
**Doses:** 4, 12, 40 mg/kg/day (M) and 4, 12, 24 mg/kg/day (F)
**Duration:** 13 weeks

Mortality: None

Clinical signs: No treatment related signs.

Bodyweight: Significant decrease in BW gain for HD males and females. At 13 weeks for males controls had gained 393 g vs 287 g for HD ($p < 0.01$). Control females gained 137 g vs 98 g for HD ($p < 0.01$).

Food consumption: There was a significant ($p < 0.05$) decrease in FC for HD males only.

Clinical Chem/Hematology: No treatment related effects.

Organ weights: Increase in rel but not absolute liver wts for HD males and females. Mean lung wts were significantly increased for HD males and females.

Gross path: Congestion of the lungs was seen in 6/10 males and 4/10 females in the HD groups. Lung congestion was not seen in the controls. There was a decrease in adipose tissue for males and females that was dose related.

Histopathology: Lungs: Increased number of alveolar macrophages, some staining for iron, in 1/10 MD and 3/10 HD females. Increased incidence of vascular congestion in HD males and females.

Liver: Increased incidence of centrilobular hepatocyte enlargement in HD males and females.

Thyroid: Slightly higher incidence of increased height of follicular epithelium associated with decreased colloid in MD and HD males.

**Sub-Acute Toxicity to Hamsters by Dietary Administration for 13 Weeks.**

**Testing facility:**
**Study number:** 9610717
**Date:** 10/96
**Species/strain:** Hamsters
**Route:** oral; mixed with diet
**Number/sex/dose:** 16/sex in control; 26/sex in treated
**Doses:** 4, 12, 40 and 60 mg/kg/day
**Duration:** 13 weeks

Mortality: One male in the HD, considered unrelated to treatment.

Clinical signs: None attributable to treatment.

Bodyweight: There was a significant decrease in bw gain in males at the LMD, HMD and HD. There was a slight, and nonsignificant decrease in bw gain in females.

Food consumption: There was a significant decrease in fc in males at the HD only.

Biochemistry: No treatment related changes.

Organ weights: There was a dose-related increase in relative lung weights for all treated males and females which was statistically significant starting at the LD. Group mean relative liver weights were increased for females.

Macroscopic Pathology: Dose related increase in lung congestion.

Histopathology: Increased incidence of lung congestion in the two highest dose gps.

Toxicokinetics: Hamsters were studied to determine if they would be a suitable species for carcinogenicity testing since rats metabolize tolterodine differently than humans. DD01 was not produced to a great extent in hamsters but at the 60 mg/kg dose, serum DD01 levels (AUC) were

39.4 (males) and 47.6 (females) ug.h/l. The concentration of DD01 was about 20% of the concentration of the parent tolterodine. Apparently the sponsors felt that this metabolite pattern was not sufficiently different from the rat to warrant use of the hamster in the carcinogenicity studies.

**Toxicity to mice by repeated oral administration for 26 weeks followed by an 8-week recovery period.**

Testing facility:
Study number: Pharmacia document 21744F
Date: 1994
Species/strain: CD-1(ICR)BR mice
Route: Oral gavage
Number/sex/dose: 30; a subgroup of 9-10 were sacrificed after 13 wks. Another gp of controls (9-10) and high dose (5-6) were treated for 26 wks then maintained for a recovery period. An additional 56/sex were treated at each dose and used for toxicokinetic sampling.
Doses: 3, 10 and 30 mg/kg
Duration: 26 weeks

Mortality: There was an increase in mortality in the high dose. Death occurred within a short time after dosing most within 1 hr. These animals had previously not shown any effects of treatment and there were no consistent abnormalities to account for the deaths and no cause of death was identified pathologically. The sponsor considers the deaths to be the result of the pharmacological action of the drug. Four HD animals died with convulsions which was considered treatment related.

Clinical signs: Convulsions prior to death in a few HD animals.

Bodyweight: Decrease in BW gain in HD females.

Food consumption: No effect.

Water consumption: Increase in water consumption in HD males and females.

Ophthalmoscopy: No effects.

Hematology: Slight but statistically significant reductions in PCV and Hb were seen for all treated female gps in comparison to controls. A decrease in PCV alone was noted for HD males. Associated with these changes were minor reductions in MCV for MD and HD animals, and increases in MCHC for HD mice. These changes were not seen after the recovery period.

Clinical chemistry: At 14 weeks, there was a slight but significant increase in BUN for MD and HD males and females and for LD males. At 26 wks, BUN was elevated in MD and HD females compared to controls but the control values were unusually low.

Urinalysis: Slight decrease in urine osmolality and sp gravity for MD and HD males. Reduction in urinary protein in HD males. Also seen in females but not significant. No differences were apparent after the recovery period.

Organ weights: Increased lung wt for HD males, increased adrenal wts for HD females and reduced liver wts for all treated female gps. All changes were reversed during recovery.
Gross path: Thickened uterus in HD females at wk 13 but not at wk 26.

Histopath: Liver - Increased incidence of centrilobular hepatocyte enlargement in HD males.

No other toxicologically significant findings were noted, including the cause of the excess deaths in the HD animals.

Summary of toxicology:

Carcinogenicity:

**24-Month Dietary Carcinogenicity Study in Mice.**

Testing facility:
Study number: N583-Q1345
Date: 1996
Species/strain: :CD-1 BR mice
Route: Oral; mixed with diet
Number/sex/dose: 60; Satellite groups of 16 were used only for determination of systemic exposure to tolterodine and its metabolite DD01.
Doses: 0, 0, 5, 15 and 30 mg/kg
Duration: 24 months.

Because of the high mortality rate observed in male mice given the MD and HD, (54% and 74%, respectively, after 76 wks of dosing), all male gps were terminated on week 79. Females were sacrificed after 24 months of study.

Mortality: Progressive dose-related mortality was observed in males given 15 and 30 mg/kg/day starting from week 41 of study. The mortality rate reached an incidence of 55% and 78% beginning at wk 78, compared to a 20% mortality rate in the control gps. No substantial differences in mortality were seen in treated females compared to controls except for a slightly higher rate in HD females (63% vs 47% in controls after 2 yrs.). The main causes of death were due to a marked distention of the large intestine due to an abnormal presence of feces and less frequently to a dilatation of the urinary bladder with marked urine retention. These effects are considered to be related to the exaggerated antimuscarinic activity of the compound.

Body weight: A slight to moderate dose-related decrease in bw gain was noted in MD and HD males and females. The mean bw of MD and HD males were about 5% and 15% lower than controls at wk 77. The MD and HD females weighed approximately 5% and 10% less than controls starting at wk 57 and these differences persisted until the end of the study (7% and 12%, respectively, on day 720).

Food consumption: Although the sponsor states that there was not a significant effect on food consumption, the data indicate that there was a slight dose related reduction in food intake for both males and females. It may be enough to explain the decrease in body weight in the treated animals.

Systemic Exposure

| Dose mg/kg | week | Tmax (hrs. of the day) | | Cmax (ng/ml) | | AUC t+20h (ngxhr/ml) | |
|---|---|---|---|---|---|---|---|
| | | M | F | M | F | M | F |
| | tolterodine | | | | | | |
| 15 | 3 | 24 | 20 | 11 | 7.3 | 159 | 92 |
| 15 | 47 | 24 | 20 | 8.7 | 6.1 | 121 | 78 |
| 15 | 78(M)/100(F) | 16 | 20 | 13 | 9.4 | 180 | 120 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 30 | 3 | 8 | 8 | 35 | 19.6 | 445 | 277 |
| 30 | 47 | 4 | 20 | 23.3 | 24.2 | 322 | 347 |
| 30 | 78(M)/100(F) | 24 | 24 | 43 | 35.4 | 367 | 375 |
| | DD01 | | | | | | |
| 15 | 3 | 4 | 20 | 6.3 | 5.2 | 107 | 80 |
| 15 | 47 | 24 | 20 | 6.9 | 5.7 | 110 | 84 |
| 15 | 78(M)/100(F) | 20 | 20 | 6.7 | 6.3 | 107 | 86 |
| 30 | 3 | 8 | 8 | 16 | 13 | 235 | 193 |
| 30 | 47 | 4 | 20 | 13.5 | 16 | 209 | 253 |
| 30 | 78(M)/100(F) | 16 | 24 | 8.6 | 12 | 129 | 171 |

The 5 mg/kg dose group had too few measurable concentrations to allow for pharmacokinetic calculations. Blood samples were taken from satellite animals at weeks 3, 47 and 78 (males)/100 (females).

Hematology: A minimal treatment-related anemia was seen in most HD males at wk 79. Possible decrease in leukocytes was seen in MD and HD males.

Clinical chemistry: No toxicologically significant changes.

Macroscopic pathology: Intestinal tract: In both MD and HD males and females there was distention of all three levels of the large intestine in those animals that died.

Microscopic pathology: Non-neoplastic lesions: Effects on intestinal tract and urinary bladder (dilation). Neoplastic lesions; No statistically significant dose tumor trends were detected in male or female mice.

**24-Month dietary Carcinogenicity Study in Rats.**

Testing facility:
Study number: N576-Q1342
Date: 1996
Species/strain: Sprague-Dawley CD(SD)BR rats
Route: Oral; mixed with diet
Number/sex/dose: 60; Satellite groups of 12 were used only for determination of systemic exposure to tolterodine and its metabolite DD01.
Doses: Males; 0, 0, 5, 15 and 30 mg/kg. Females 0, 0, 5, 10 and 20 mg/kg.
Duration: 24 months.

Mortality: No significant difference in mortality was seen between controls and treated animals of either sex. After 2 yrs the incidence of mortality in controls was 36% in males while it ranged from 43% to 52% in females. In treated gps the mortality rate ranged from 28% to 43% in males and from 43% to 60% in females without a dose relationship in either sex. Deaths in some incidences was due to distention of the large intestine due to an abnormal presence of feces.

Body weight: a dose-related decrease in bw gain, compared to controls, was observed at the MD and HD in both sexes. In males the bw gain was 20% to 35% less than controls at the end of the study. In females the decrease in bw gain was 28% and 44% in MD and HD, respectively, at the end of the study.

Food consumption: A 10% decrease in food intake was seen in HD males throughout the study. In females there was a 10% reduction in the MD and a 15% reduction in the HD compared to

controls up to one year of treatment. After about week 76, the mean food intake in the MD and HD females was similar to that of controls.

Hematology: No treatment related changes.

Clinical Chemistry: No toxicologically significant changes.

Urinalysis: No treatment related changes.

Systemic Exposure: Tolterodine

| Dose (mg/kg/day) | Week | Cmax (ng/ml) | | AUC (ngxh/ml) t + 20h | |
|---|---|---|---|---|---|
| | | Males | Females | Males | Females |
| 5 | 3 | 0.53 | 0.27 | 4.2 | — |
| 5 | 53 | 0.41 | 0.64 | 6.0 | 7.4 |
| 5 | 103 | 3.9 | 2.2 | 44 | 23 |
| 15(M)/10(F) | 3 | 1.3 | 1.2 | 20 | 17 |
| 15(M)/10(F) | 53 | 3.9 | 6.3 | 42 | 95 |
| 15(M)/10(F) | 103 | 74.9 | 4.8 | 764 | 70 |
| 30(M)/20(F) | 3 | 3.2 | 16 | 44 | 217 |
| 30(M)/20(F) | 53 | 97 | 28 | 623 | 388 |
| 30(M)/20(F) | 103 | 73 | 17 | 718 | 269 |

Exposure to metabolite DD01 was negligible as no or very low serum levels of DD01 were detected.

Macroscopic Pathology: Intestinal tract; marked distention of the large intestine was seen in one LD male, two MD males and one MD female and nine males and seven females from the high dose group. This antimuscarinic effect was related to the deaths of these animals and the intestinal distention also occurred in one MD male that survived to the end of the study.

Lungs: Increased incidence of abnormal colored areas was seen in HD males and females.

Histopathology: The changes noted macroscopically in the large intestine were obscured by autolysis.

Lungs: Increased incidence and severity of alveolar macrophages was seen in HD males which correlated with the colored areas seen macroscopically.

Neoplastic findings: There was a significant dose-tumor positive linear trend for malignant renal liposarcoma in male rats and for benign vaginal fibromas in females.

Immunotoxicology:

## Reprotoxicology:

The preliminary reproduction studies were sponsors summaries.

**Preliminary segment I study in the male mouse**

Experimental design
The influence of tolterodine upon reproductive function and fertility was assessed in a preliminary study in sexually mature male mice of the CD-1 strain.

Tolterodine was administered by gavage at dosages of 12, 20 or 40 mg/kg/day to groups of six or ten male mice for 15 days before pairing. Treatment of the males was continued until the untreated females had littered. After delivery the offspring were reared until day 4 after birth. Control males received the vehicle, water, throughout the same period.

Results

Three males, treated with tolterodine at a dosage of 40 mg/kg/day, exhibited postdosing piloerection during the first three days of treatment. With these exceptions, the general condition of the animals was similar in all groups. One male receiving 40 mg/kg/day was found dead after dose administration on the first day of treatment. Body weight and bodyweight gain of the males were unaffected by treatment with tolterodine.
Food intake of the males was unaffected by treatment.
Water intake of the males was slightly increased in the 40 mg/kg/day dosage group. Mating performance and fertility were unaffected by treatment.
Littering and litter parameters were unaffected by treatment of the parental males. Necropsy findings for the parents and their offspring did not reveal any changes considered to be attributed to treatment.

Conclusions

It was concluded from this dose range-finding study, in the male mouse, that dosages of tolterodine of up to 40 mg/kg/day, administered by oral gavage, should be suitable for use in a main fertility study in this strain of animal.

**Preliminary segment I and III study in the female mouse**

Experimental design
The influence of tolterodine upon reproductive function and fertility was assessed in a preliminary study in sexually mature female mice of the CD-1 strain.

Tolterodine was administered by gavage at dosages of 4, 12, 20 or 30 mg/kg/day to groups of six female mice for 15 days before pairing. Treatment of the females was continued throughout mating, gestation and lactation to Day 7 after birth. Control females received the vehicle, water, throughout the same period.

Results

The general condition of the parental females was similar in all groups and no deaths occurred. Body weight performance was slightly inferior in animals treated with 30 mg/kg/day, compared with the control group, during the last week of the gestation period.

Food intake was slightly reduced in the 30 mg/kg/day treatment group from the second week of treatment compared with the control group. In the same treatment group water intake was decreased during the lactation period.
Estrous cycles, mating performance and fertility, and littering were unaffected by treatment.
The number of implantation sites, litter size and post-implantation survival index were reduced in females treated with 30 mg/kg/day compared with the control group. Other litter parameters were unaffected by treatment.
Necropsy findings for the parental females and offspring did not suggest any treatment-related findings.

Conclusion

It was concluded from this dose range-finding study, in the female mouse, that dosages of up to 20 mg/kg/day, administered by oral gavage, should be suitable for use in a main fertility study in this strain of animal.

**Reproductive function and fertility study in the mouse (segment I).**

Testing facility:
Study number: 94/KBH008/0389
Date: 1994
Species/strain: CD-1 mice
Route: oral gavage
Number/sex/dose: 40
Doses: Males: 3, 10 and 30 mg/kg/day. Females: 3, 10 and 20 mg/kg/day
Duration: Males: from 71 days before pairing until successful parturition. Females: from 15 days before pairing, throughout the mating period and during gestation and lactation. Up to 26 females from each gp were killed on day 17 of gestation for examination of uterine contents. The remaining females were allowed to give birth and rear their young to weaning at day 22 of lactation. The offspring were then examined for behavioral responses.

Clinical signs: None treatment related.

Mortality: Six total, 3 in HD group, one male found dead on day 2 with no abnormalities at necropsy. Death possibly due to pharmacological effect of drug. All other deaths unrelated to treatment.
Bodyweight: No effect in males. In females, bw gain was marginally less in HD from day 12 of gestation to birth when compared to controls.

Mating performance and fertility: No treatment effects.

Teratology phase; females killed on day 17 of gestation
Mean no. of corpora lutea was significantly decreased in HD females and as a result there was a decrease in the number of implantations and viable young. The number of early and total resorptions were higher in HD when compared to controls and as a consequence, the extent of post-implantation loss was greater but not statistically significant. Fetal weight in HD gp was slightly lower than controls but not significant and within control range and not thought to be due to treatment.

Fetal evaluation: No treatment related abnormalities.

Post-natal phase:

No effect on gestation length, parturition, gestation index, general condition of offspring, litter size and survival, physical development, auditory and visual responses, locomotor activity, water maze performance and sex ratio.

Bodyweight of offspring: At day 1 of age, bodyweights of male and female offspring of HD gp were slightly lower than those of controls. Subsequent bodyweights and bw gain were also low in this gp compared to controls. No other gps were affected.

F1-F2 generation
No treatment related effects on any parameter.

**A preliminary study of the effect of 2234 on pregnancy of the mouse**

Experimental design

In this preliminary assessment of the effect of tolterodine on pregnancy in the mouse, dosages of 0, 10, 30 and 90 mg/kg/day were administered orally to 12 female mice per group from Days 6 to 15 of pregnancy inclusive. On Day 17.5 the females were sacrificed, subjected to macroscopic post mortem examination, litter values determined and fetuses examined for gross abnormalities.

Results

In the parent females reduced bodyweight gain and slightly reduced food consumption were noted among dams given 90 mg/kg/day reflecting the lack of viable fetuses at this dose level. No effects of treatment on body weight gain and food consumption were noted among dams given 10 or 30 mg/kg/day.

Administration of 90 mg/kg/day resulted in total resorption of litters in all pregnant females. Administration of 10 or 30 mg/kg/day had no effect on the incidence of total resorption.

Among the surviving litters at termination there were reductions in mean fetal weight and total litter weight in the groups given 10 or 30 mg/kg/day in comparison with controls; however, no dosage-related trend was apparent. There was no indication of a treatment-related effect on the incidence of gross morphological changes noted.

Conclusions

Embryo viability was completely compromised among animals receiving 90 mg/kg/day. In addition mean fetal weights were reduced at both dosages where litters were produced (10 or 30 mg/kg/day). In view of these effects it is considered that further preliminary investigations are needed to establish suitable dosages for embryo-fetal toxicity studies.

**Preliminary study of the effect of 2234 on pregnancy of the mouse**

Experimental design

In this second preliminary assessment of the effect of tolterodine on pregnancy in the mouse, dosages of 0, 5, 15, 45 and 70 mg/kg/day were administered orally to 12 female mice per group from Days 6 to 15 of pregnancy inclusive. On Day 17.5 the females were sacrificed, subjected to macroscopic post mortem examination, litter values determined and fetuses examined for gross abnormalities.

Results

In the parent females reduced bodyweight gain and slightly reduced food intake were noted among females given 70 or 45 mg/kg/day reflecting the lack of viable fetuses at these dose levels. No effects on body weight gain and food consumption were noted among females treated at 5 or 15 mg/kg/day.

Administration of 70 mg/kg/day resulted in total resorption of litters in all pregnant animals. Administration of 45 mg/kg/day resulted in total resorption of 2/10 litters, whereas no total litter loss was recorded in controls or females given 5 or 15 mg/kg/day.

Among surviving litters of dams given 45 mg/kg/day there were reductions in mean fetal weight and litter weight with clear increase in post implantation loss. There were no apparent adverse effects of treatment at 5 or 15 mg/kg/day. There was no indication of a treatment-related effect on the incidence of gross morphological changes noted.

Conclusion

There were clear effects on embryofetal parameters, embryo viability being completely compromised at 70 mg/kg/day and reduced at 45 mg/kg/day, with litter and mean fetal weights being reduced at 45 mg/kg/day. In view of the above, dosages of 45 mg/kg/day and above are considered unsuitable for a main embryofetal toxicity study.

**The effect of Tolterodine on the pregnancy of the mouse following oral administration**

Testing facility:
Study number: document 21037F
Date: 1993
Species/strain: CD-1 mice
Route: Oral gavage
Number/sex/dose: 30 females with 24 additional females used for toxicokinetic monitoring
Doses: 4, 12 and 40 mg/kg/day
Duration: From day 6 to day 15 of pregnancy

Clinical signs and mortality: No clinical signs. No treatment related deaths.

Bodyweight: Bodyweight gains in HD were lower than controls from day 10 of pregnancy with differences from controls attaining statistical significance. This was considered a consequence of the reduced litter weight.

Food consumption: No effects.

Gross pathology: None.

Litter data

A total of 1/25, 0/26, 1/24 and 9/29 pregnant females (control to HD, respectively) resorbed their litters pre-term. The females that showed total resorption in control and LD had few implantations and showed weight loss from the start of treatment. These resorptions were considered coincidental since it is not unusual for females with only a few implantations to show total resorption.

For the HD females, 3 instances of resorption were similar to the two cases above. However, for the remaining 6, the number of implantations was higher and effects on bodyweight were often

apparent at later stages of pregnancy. Late in utero deaths were apparent in 2 females. It was concluded that these resorptions were due to treatment although it is uncertain if there was an indirect effect on the dams or if there was a direct in utero effect upon the conceptus.

Litter size, embryonic losses and sex ratio

No obvious treatment effect on implantations (which generally occur prior to treatment), although the number of implantations were lower in the treated gps than in the controls.

In utero deaths

| Category | Total number in Group | | | |
|---|---|---|---|---|
| All pregnant females | control | LD | MD | HD |
| Implantations | 303 | 277 | 269 | 310 |
| In utero deaths | | | | |
| early | 22 | 14 | 22 | 93 |
| late | 3 | 0 | 2 | 54 |
| Females with live young | | | | |
| Implantations | 300 | 277 | 267 | 236 |
| In utero deaths | | | | |
| early | 19 | 14 | 20 | 36 |
| late | 3 | 0 | 2 | 37 |

There was no effect on sex ratio

Litter and mean fetal weight

Litter and mean fetal weight were significantly reduced at the HD when compared to controls. There were no obvious treatment related effects at the lower doses.

Malformations, anomalies and variants

| Number of affected fetuses per litter | Number of litters in group | | | |
|---|---|---|---|---|
| Malformation | control | LD | MD | HD |
| 0 | 20 | 21 | 23 | 9 |
| 1+ | 4 | 5 | 0 | 11 |
| Visceral anomaly | | | | |
| 0 | 19 | 24 | 19 | 13 |
| 1+ | 4 | 2 | 4 | 2 |
| 2+ | 1 | 0 | 0 | 4 |
| Skeletal anomaly | | | | |
| 0 | 18 | 18 | 16 | 2 |
| 1+ | 3 | 5 | 4 | 10 |
| 2+ | 3 | 3 | 2 | 7 |

There was a clear increase in the number of litters with one or more fetuses with structural changes (malformations and anomalies) at the high dose.

At 40 mg/kg/day a higher number of fetuses were considered malformed, with principal changes attributable to treatment being:

| malformation | control | high dose |
|---|---|---|
| cleft palate | 0 | 10 |
| digital abnormalities | 1 | 7 |
| intra-abdominal hemorrhage | 2 | 6 |
| reduced ossification | 8 | 17 |
| irregular ossification of costal cartilage elements | 1 | 10 |
| fused/connected sternebrae | 0 | 3 |
| additional thoraco-lumbar vertebra | 0 | 6 |

**A preliminary study of the effect of 2234 on the pregnant and non-pregnant rabbit**

Experimental design

This study was a preliminary assessment of the effect of tolterodine on the pregnant and non-pregnant rabbit. Eight animals were employed. The study was performed in two phases.

Phase I consisted of two non-pregnant female animals, treated via the oral route with single doses of 8, 24, 72 and 48 mg/kg on Days 1, 4, 7 and 10 respectively.

Phase II consisted of two non-pregnant female animals, treated via the oral route at a dosage of 12 mg/kg/day for 13 days, and four pregnant animals treated by subcutaneous injection at varying doses including 0.2, 1, 3 and 5 mg/kg according to the schedule below. Dosing of pregnant rabbits commenced on Day 6 of pregnancy and finished, in the surviving rabbit, on Day 18 of pregnancy.

Results

Phase I

8 mg/kg resulted in increased respiration in both rabbits and transient bodyweight loss and reduced food consumption in 1/2 rabbits.

24 mg/kg resulted in increased respiration, transient bodyweight loss and reduced food consumption.

72 mg/kg resulted in increased respiration and unsteady gait in both rabbits, dilated pupils, convulsions and death in 1/2 and dark eyes in the survivor. Transient bodyweight loss and reduced food consumption were noted in the survivor.

48 mg/kg resulted in increased respiration and dark eyes, transient bodyweight loss and continuous reduced food consumption up to sacrifice 5 days later.

There were no gross macroscopic pathological abnormalities considered to be related to treatment in either rabbit.

Phase II - non-pregnant rabbits

Treatment of 2 non-pregnant female rabbits with 12 mg/kg/day orally for 13 days resulted in no clinical signs or deaths, bodyweight loss for the first 2 days of treatment followed by fluctuating losses and gains, and reduced food consumption for the first 12 days of treatment. There were no gross macroscopic pathological abnormalities noted at necropsy and no abnormalities noted at histopathological examination of livers that were considered to be related to treatment

Phase II - pregnant rabbits

Results of determination of parent drug serum levels following oral administration in Phase I of the study (data on file at the Sponsor) indicated that very low levels of parent drug were achieved. Thus it was decided to investigate the tolerance following a parenteral route of administration (subcutaneous).

Subcutaneous dosing of pregnant rabbits according to the following schedule gave these results:

| Animal number | Dose level (mg/kg) | Days of pregnancy dosed | Findings |
|---|---|---|---|
| | 1 | 6-8 | Dilated pupils and increased respiration after all doses, convulsions and death after the third dose; bodyweight loss; reduced food consumption; no gross macroscopic abnormalities |
| | 1 | 6 | No clinical signs; bodyweight loss; no effect on food consumption. |
| | 3 | 7, 8 | Increased respiration; bodyweight loss; marked reduction in food consumption. |
| | 0.2 | 11-18 | Increased respiration after first dose only, then no clinical signs; no effect on bodyweight or food consumption. |
| | Sacrificed Day 29 | | No gross macroscopic abnormalities in dam or pups attributable to treatment. No effect on litter parameters attributable to treatment. |
| 5 | | 6 | Increased respiration, dilated pupils, dark eyes, found dead. No post dosing bodyweight or food consumption data available. No gross macroscopic abnormalities. |
| 5 | | 6 | Increased respiration, dilated pupils, dark eyes, 1 convulsion; marked bodyweight loss and reduced food consumption. |

| | | |
|---|---|---|
| 3 | 7 | Increased respiration, 3 convulsions, died following third convulsion; No post-dosing bodyweight or food consumption data available; no gross macroscopic abnormalities attributable to treatment. |

Overall, the spectrum of clinical signs noted was typical of those associated with the anticholinergic activity of the compound. The deaths were also likely to have resulted from this pharmacological effect. At sub-lethal doses, reduced growth and food consumption indicated that treatment with the test compound was compromising the health status of the animals. However no treatment-related macroscopic pathological change was evident in any of the animals.

Conclusions

The dose range finding for oral administration carried out as Phase I of this study enabled the selection of 12 mg/kg/day as a dose level for repeated administration. This dosage level in non-pregnant female rabbits produced initial bodyweight loss and reduced food consumption. It is considered that the highest oral dose that should be given to pregnant rabbits is in the region of 12 mg/kg/day.

Subcutaneous doses of 1, 3 and 5 mg/kg resulted in death and are clearly unsuitable for further investigation. A dose level of 0.2 mg/kg/day subcutaneously from Days 11 to 18 of pregnancy had no substantial adverse effects. It is considered that a high dosage level of above 0.2 but less than 1 mg/kg/day would be suitable for further investigation of the effects of tolterodine by the subcutaneous route in the pregnant rabbit.

**A preliminary study in the pregnant rabbit with toxicokinetic monitoring.** **2234 by gavage together with**

Experimental design

In this preliminary assessment of the effect of tolterodine on pregnancy in the rabbit, dosages of 0, 4, 8 and 15 mg/kg/day were administered orally to 8 rabbits per group from Days 6 to 18 of pregnancy inclusive. On Days 6 and 16 samples were obtained for serum drug level analysis. On Day 29 the animals were sacrificed, subjected to a post mortem examination, litter values determined and fetuses examined for gross abnormalities.

Results

In the parent females increased respiration was observed post-dosing on the first two days of treatment in a few animals receiving 8 or 15 mg/kg/day. A reduction in food intake was noted at 15 mg/kg/day.

No treatment-related effect on body weight gain was noted.

There were no adverse effects of treatment of the litter parameters assessed.

Analysis of serum concentrations of tolterodine on days 6 and 16 of pregnancy showed a mean peak serum level (Cmax) of 1.5 ug/l after treatment with 4 mg/kg/day. After treatment with 8 mg/kg/day the mean Cmax value was about 2 ug/l and at 15 mg/kg/day the corresponding value was about 6 ug/l. Estimation of the area under the serum concentration curve (AUC) for

tolterodine showed values of about 5 ugxh/l after 4 mg/kg/day, 9 ugxh/l after 8 mg/kg/day and 25 ugxh/l after 15 mg/kg/day. Analysis of the hydroxylated metabolite of tolterodine (DD01) showed mean peak serum levels of about 1 ug at 4 mg/kg/day and 1-2 ug/l at 8 mg/kg/day. After treatment with 15 mg/kg/day the mean Cmax value was about 6 ug/l. AUC values for DD01 were about 5, 8 and 20 ugxh/l for the three dosage levels.

Conclusions

In the parent females post-dosing clinical sign and reduced food intake were recorded at 15 mg/kg/day. There were no effects of treatment on embryofetal parameters. In view of the above a dosage in the region of 15 mg/kg/day is considered a suitable top dosage for an ensuing embryo-fetal toxicity study using the oral route.

**A preliminary study in the pregnant rabbit with 2234 by subcutaneous injection together with toxicokinetic monitoring**

Experimental design

In this preliminary assessment of the effect of tolterodine on pregnancy in the rabbit, dosages of 0, 0.2, 0.45 and 0.9 mg/kg/day were administered via subcutaneous injection to 8 rabbits per group from days 6 to 18 of pregnancy inclusive. On days 6 and 16 samples were obtained for serum drug level analysis. On day 29 surviving animals were sacrificed, subjected to a post mortem examination, litter values determined and fetuses examined for gross abnormalities.

Results

In the parent females, one rabbit given 0.9 mg/kg died following the first dose. Also, weight losses and reduced food intake were noted on the first two days of treatment at 0.9 mg/kg/day.

Weight loss over the first 2 days of treatment and reduced food intake for the first 8 days of treatment were noted at 0.45 mg/kg/day and reduced weight gain over the first 2 days of treatment only at 0.2 mg/kg/day.

There were no adverse effects of treatment on the litter parameters assessed.

Analysis of serum concentrations of tolterodine on days 6 and 16 of pregnancy showed a mean peak serum level (Cmax) of about 10 ug/l after treatment with 0.2 mg/kg/day. After treatment with 0.45 mg/kg/day the mean Cmax value was about 20 ug/l. At 0.9 mg/kg/day the mean Cmax value was about 40 ug/l. Estimation of the area under the serum concentration curve (AUC) for tolterodine showed values of about 25 ugxh/l after 0.2 mg/kg/day and about 110 ugxh/l after 0.9 mg/kg/day. Analysis of the hydroxylated metabolite of tolterodine (DD01) showed mean peak serum levels of 0.7 ug/l at 0.2 mg/kg/day and 1.3 ug/l at 0.45 mg/kg/day. After treatment with 0.9 mg/kg/day the mean Cmax value was about 4 ug/l. AUC values for DDO 1 were about 6 ugxh/l at 0.2 mg/kg and 20 ugxh/1 at 0.9 mg/kg/day.

Conclusions

One treatment related death occurred after dosing at 0.9 mg/kg. In the parent females at all dosages of tolterodine (0.2, 0.45 and 0.9 mg/kg/day) there was a transient initial adverse effect on body weight gain.
There were no effects of treatment on embryofetal parameters.
A dose level slightly below 0.9 mg/kg/day is considered suitable for an ensuing embryo-fetal toxicity study using the subcutaneous route.

**A study of the effect of tolterodine on pregnancy of the rabbit following subcutaneous administration.**

Testing facility:
Study number: document 21036F
Date: 1993
Species/strain: New Zealand White Rabbits
Route: subcutaneous injection
Number/sex/dose: 16 females/dose
Doses: 0.2, 0.4 and 0.8 mg/kg/day
Duration: From day 6 to day 18 of pregnancy.

Clinical signs and mortality: One HD rabbit was found dead 2 hrs after dosing on the third day of treatment. Post mortem exam did not reveal the cause of death but the sponsor considered it treatment related.

Bodyweight: Initial treatment related bodywt loss of -2, -16 and -17 g compared to a +12 in the control gp by day 8. This was not statistically significant and was not sustained.

Food consumption: Statistically significant reduction in food intake at the HD during first few days and consumption remained slightly less until day 13 of pregnancy. Following cessation of treatment on day 18, food consumption of these rabbits was greater than that of controls from day 19 to day 28.

Terminal sacrifice: No differences noted.

Litter parameters

No obvious treatment related effects on the number of corpora lutea, implantation losses, type and distribution of embryonic deaths, live young, litter weight, mean pup weight and sex ratio.

Malformations, anomalies and skeletal variants

A slightly higher percentage incidence of gross/visceral anomalies was noted in HD rabbits (13.2%) in comparison with controls (5.8%) although this difference was not statistically significant. This was due to a greater incidence in the HD of anomalous cervico-thoracic arteries or abnormal lung lobation. The sponsor states that the incidence of anomalous cervico-thoracic arteries was within the control background range (6/116 fetuses affected in this study compared to a range of 0/109 to 10/112 in controls of 13 studies conducted around the same time). The anomalous lung lobes was above control background range (4/116 compared to 0/277 to 2/145) in the same 13 studies but the sponsor dismisses the result since 3 out of 4 of the affected fetuses were in the same litter and the number of litters affected was similar to the background range (2/14 compared to 0/31 to 2/18).

Note: For the anomalous cervico-thoracic arteries, need to know the historical range for the number of litters affected since in this study there were 2/16, 2/14, 4/16 and 6/14 in the control, LD, MD and HD respectively. There were no increase in cleft palate or any of the visceral or skeletal anomalies seen in mice.

## Genotoxicology: Sponsor's summaries

**Study to determine the ability of tolterodine to induce mutation in two tryptophan requiring strains of Escherichia coli.**

Tolterodine was assayed for mutation in 2 tryptophan-requiring strains (WP2 pKM101 and WP2 uvrA pKM101) of Escherchia coli, both in the absence and presence of metabolic activation by an induced rat liver post-mitochondrial fraction (S-9), In 2 separate experiments.
Experiment 1 treatments were carried out with both test strains, using final concentrations of tolterodine at 8, 40, 200, 1000 and 5OOO ug/plate plus a solvent and positive control. These treatments resulted in toxicity to strain WP2 uvrA pKM101 at 5OOO ug/plate, with further evidence of toxicity, in the form of a thinning of the background bacterial lawn, at 1000 ug/plate. 5OOO ug/plate treatments of strain WP2 PKMIOI produced varying signs of toxicity, but in all cases some evidence of toxicity was observed.
For Experiment 2, the maximum test treatments for strains WP2 pKMI01 and WP2 uvrA pKMI01 were reduced to 25OO and 1000 mg/plate respectively, these doses being estimates of the limits of toxicity for the 2 strains. For each strain a narrowed dose range was utilized in order to more closely examine those doses which were most likely to exhibit a mutagenic response. Experiment 2 treatments in the presence of S-9 also incorporated a pre-Incubation step, and in this way It was hoped to increase the range of mutagenic chemicals that can be detected using this assay system. Following Experiment 2 treatments evidence of slight toxicity, in the form of a thinning of the background bacterial lawn, and/or a marked reduction in reverent numbers below that of the solvent control treatments, was observed on all plates treated at the maximum done for each strain.
Negative (solvent) and positive control treatments were included for all strains In both experiments. The mean numbers of reverent colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.
Following treatments of the test strains In the absence and presence of metabolic activation (S-9), no statistically significant or dose related increases in reverent numbers were observed. This study therefore provided no evidence of tolterodine mutation induction.

It Is concluded that tolterodine was unable to induce mutation in 2 strains of Escherichia coli, when tested at concentrations up to the limit of toxicity In both the absence and presence of a rat liver metabolic activation system.

**Study to determine the ability of tolterodine to induce mutation in four histidine-requiring strains of Salmonella typhimurium.**

Tolterodine was assayed for mutation in four histidine requiring strains (TA98, TA100, TA1535 and TA1537) of Salmonella typhimurium, both in the absence and presence of metabolic activation by an induced rat liver postmitochondrial fraction (S-9), in two separate experiments.
An initial toxicity range-finder experiment was carried out in TA100 only, using final concentrations of tolterodine at 8, 40, 200, 1000 and 5000 ug/plate plus a solvent (dimethyl sulphoxide) and positive control. Complete toxicity was observed at 5000 ug/plate in the absence and presence of S-9. In experiment 1, therefore, the top test dose was reduced to 2500 ug/plate for all treatments, with a five-fold dilution series again providing the remaining doses. Despite these alterations, some toxicity was observed at 2500 ug/plate with strains TA98 and TA1535 in the absence of S-9, and both in the absence and presence of S-9 with TA1537. In the light of experiment 1 results, in experiment 2 the top dose for treatments in the absence of S-9 was reduced to 2000 ug/plate, whilst the top dose for treatments in. the presence of S-9 was maintained at 2500 ug/plate. In addition to these alterations, narrowed dose ranges were utilized in experiment 2 in order to investigate more closely those concentrations of tolterodine most likely to exhibit a mutagenic response. Following experiment 2 treatments, some signs of toxicity were again observed

following top dose treatments of strains TA98, TA100 and TA1537 in the absence of S-9, and of strains TA100 and TA1535 in the presence of S-9.
Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of reverent colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.
No treatment of any of the four test strains induced a significant increase in reverent numbers. In no case was there a two-fold (TA98 and TA100) or three-fold (TA1535 and TA1537) increase in reverent numbers that would normally be sufficient for a clear induction of mutation in these strains.

It is concluded that tolterodine was unable to induce mutation in four strains of Salmonella typhimurium, when tested up to concentrations toxic to the test bacteria in the absence and presence of a rat-liver metabolic activation system.

**Study to evaluate the chromosome damaging potential of tolterodine by its effects on cultured human lymphocytes using an in vitro cytogenetics assay.**

Tolterodine was tested in an in vitro cytogenetics assay using duplicate human lymphocyte cultures from a single female donor. Treatments were performed both in the absence and presence of metabolic activation by a rat liver postmitochondrial fraction (S-9) from
induced animals. The test compound dose levels for analysis were selected by determining mitotic indices from a broad range of doses up to 1700 ug/ml, a concentration which induced toxicity and which was close to the maximum achievable practically using dimethyl sulphoxide as solvent. The top dose considered suitable for analysis, 466.9 ug/ml, induced approximately 55% and 81% mitotic inhibition in the absence and presence of S-9 respectively. The doses analyzed for chromosome aberrations were 197.2, 303.5 and 466.9 ug/ml. Appropriate negative (solvent) control cultures were included in the test system and contained low incidences of chromosomal aberrations within historical solvent control ranges. Methyl methanesulphonate (MMS) and cyclophosphamide (CPA) were employed as positive control chemicals in the absence and presence of liver S-9 respectively. Both compounds induced statistically significant increases in the incidence of chromosomal aberrations.
Treatment of cells in the absence and presence of S-9 resulted in numbers of structural aberrations which were similar to and not significantly different from those observed in concurrent solvent controls. Historical solvent control ranges for structural aberrations were not exceeded at any of the treatment doses analyzed. When gaps and/or numerical aberrations were included in the data, aberration frequencies in one culture treated with the intermediate dose and one culture treated with the low dose in the absence of S-9 exceeded historical control ranges. This was not considered to be of biological significance as increases were small and were not observed in the replicate cultures nor at the higher dose.

It is concluded that was unable to induce structural chromosome aberrations in cultured human lymphocytes when tested to its limit of toxicity in either the absence or presence of S-9.

**Study to evaluate the chromosome damaging potential of tolterodine by its effects on cultured human lymphocytes using an in vitro cytogenetics assay.**

Tolterodine was tested in an in vitro cytogenetics assay using duplicate human lymphocyte cultures from a male donor. The highest dose level used, 700 ug/ml was based on data generated in a previous assay on this chemical (above). Treatments covering a broad range of doses, separated by narrow intervals, were performed both in the absence and presence of metabolic activation by a rat liver post-mitochondrial fraction (S-9) from induced

animals. Treatment in the absence of S-9 was continuous for 20 or 44 hours (20+0, 44+0). Treatment in the presence of S-9 was for 3 hours only followed by a 17 or 41 hour recovery period prior to harvest (3+17, 3+41). An additional treatment, in the absence of S-9, for 3 hours followed by a 17 hour recovery period was also performed.
The dose levels for analysis at each sampling time were selected by evaluating the effect of tolterodine on mitotic index. Chromosome aberrations were analyzed in cells receiving 20+0 hour treatments in the absence of S-9 and 3+17 hour treatments in its presence at 3 consecutive dose levels. The highest concentrations chosen for analysis were, 117.1 ug/ml (20+0, - S-9) and 505.8 ug/ml (3+17, + S-9), which induced approximately 67% and 63% mitotic inhibition respectively. The effects of single concentrations only, 99. 57 and 505.8 ug/ml (without and with S-9) were investigated at the delayed (44 hour) sampling time and following the 3+17 hour treatment in the absence of S-9 (429.9 ug/ml).
Appropriate negative (solvent) control cultures were included in the test system under each treatment condition. The proportion of cells with structural aberrations in these cultures fell within historical solvent control ranges. 4-Nitroquinoline 1-oxide (NQO) and cyclophosphamide (CPA) were employed as positive control chemicals following 20+0 hour treatments in the absence and 3+17 hour treatments in the presence of liver S-9 respectively. Both compounds induced statistically significant increases in the proportion of cells with structural aberrations.
In an initial experiment (consisting of 2 trials) several solvent control cultures were observed to have frequencies of cells with aberrations which exceeded historical solvent control ranges, thus data from this aborted experiment (both trials) are not reported here.
Treatment of cultures with tolterodine under most exposure conditions in the absence of S-9 resulted in frequencies of cells with chromosomes aberrations which were similar to and not significantly different from those in concurrent solvent controls. A small, but statistically significant increase in aberrations at the intermediate dose level scored following 20 hours of treatment was considered unlikely to be biologically significant insofar as it was not seen at the higher dose although mitotic inhibition (cell cycle delay) at both doses was similar.
Treatment of cultures in the presence of S-9 gave frequencies of aberrant cells which were significantly higher than those in concurrent controls at the low dose level scored following the 3+17 hour treatment (365.4 ug/ml) and at 505.8 ug/ml following the 3+41 hour treatment. These increases were not considered biologically significant because numbers of aberrant cells in all cultures fell within the normal range.

It is concluded that tolterodine was unable to induce chromosome aberrations in human lymphocytes when tested to its limit of toxicity in both the absence of and presence of S-9.

**Study to determine the ability of tolterodine to induce mutations at the locus in mouse lymphoma L5178Y cells using a fluctuation assay.**

Tolterodine was assayed for mutation at the locus (6-thioguanine resistance) in mouse lymphoma L5178Y cells using a fluctuation protocol. The study consisted of two independent experiments, each conducted in the absence and presence of metabolic activation by an induced rat liver post-mitochondrial fraction (S-9).
Following a wide range of treatments, separated by half-log intervals and reaching 2160 ug/ml, cells survived at 216 ug/ml yielding 26% relative survival in the absence and 83% relative survival in the presence of S-9. This dose, together with the next 4 lower doses, was plated for viability and 6-thioguanine resistance 7 days after treatment. In the second experiment a narrower dose range was used to maximize the chance of detecting any dose-related effects. The top doses plated in this experiment were 250 ug/ml and 500 ug/ml in the absence and presence of S-9, which yielded 69% and 3% relative survival respectively. In this experiment these and the next 3 (- S-9) or 4 (+ S-9)- lower doses were plated for determination of mutant frequency 7 days after treatment.

Solvent (DMSO) and positive control treatments were included in each experiment in the absence and presence of S-9. Mutation frequencies in negative (solvent) control cultures fell within normal ranges, and statistically significant increases in mutation were induced by the positive control chemicals 4-nitroquinoline l-oxide (without S-9) and benzo(a)pyrene (with S-9).
In both experiments, no treatment with tolterodine, either in the absence or presence of S-9, resulted in a statistically significant increase in mutation frequency.

It is concluded that, under the conditions employed in this study, tolterodine failed to induce mutation at the locus of L5178Y mouse lymphoma cells when tested up to toxic concentrations, in the absence and presence of a rat liver S-9.

**Study to evaluate the potential of tolterodine to induce micronuclei in the polychromatic erythrocytes of CD-1 mice.**

Tolterodine was assayed in vivo in a mouse bone marrow micronucleus test at 3 dose levels. The choice of dose levels was based on an initial toxicity range-finder study in which tolterodine dissolved in distilled water was administered orally to 3 male and 3 female mice on 2 consecutive days at doses of 105.5, 140.6, 187.5 and 250 mg/kg. From the pattern of mortality the LD50 was estimated at approximately 217 mg/kg (x 2). For the micronucleus test tolterodine was dissolved in distilled water and administered orally as 2 daily doses at 37.5, 75 and 150 mg/kg to groups of 5 male and 5 female mice killed 24 or 48 hours after the second dose. Two animals receiving the highest dose died prior to sampling indicating that it would not have been practicable to administer the test chemical at a higher dose.
The negative (vehicle) control in the study was distilled water also administered orally on 2 consecutive days. Groups of 5 male and 5 female mice treated with this were killed and sampled 24 or 48 hours after the second dose. Cyclophosphamide (CPA), the positive control, was dissolved in water and administered orally as a single dose at 80 mg/kg to groups of 5 male and 5 female mice which were killed after 24 hours. All positive control animals exhibited increased numbers of micronucleated polychromatic erythrocytes (PCE) such that the micronucleus frequency in the positive control group was significantly greater than in the negative control group. Slides from all dose groups sacrificed after 24 hours, and slides from top dose and control groups sacrificed after 48 hours were analyzed. Negative (vehicle) control mice exhibited acceptable ratios of PCE to NCE (normochromatic erythrocytes) and normal frequencies of micronucleated PCE within historical negative control ranges. Mice treated with tolterodine at all doses and sampling times exhibited ratios of PCE to NCE and frequencies of micronucleated PCE which were similar to vehicle controls. There were no instances of statistically significant increases in micronucleus frequency for any of the groups receiving the test chemical at any sampling time.

It is concluded that tolterodine did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice treated on two consecutive days with doses up to 150 mg/kg, a dose at which limited mortality was observed.

**Genotoxicology of DD01**

The active metabolite DD01 was tested separately in in vitro tests using
and human lymphocytes with and without a S-9 metabolizing system. The results were negative.

Special toxicology studies:

## OVERALL SUMMARY

Tolterodine is a competitive muscarinic receptor antagonist. It binds with high affinity and specificity to muscarinic receptors, as compared with other receptor types. It is more potent in inhibiting acetylcholine-induced urinary bladder contractions than electrically-induced salivation in vivo in anesthetized cats.

Tolterodine has been adequately tested in mice, rats and dogs. Most of the effects seen were the result its antimuscarinic actions. There was increased motor activity, mydriasis, decreased intestinal motility, development of residual urine and increased heart rate. Some dogs given 4.5 mg/kg showed ataxia, sedation and sensitivity towards light and trembling. In these dogs, there was a higher frequency of keratitis and/or corneal opacities and conjunctivitis compared to controls. The pupillary reflex was absent in some high dose dogs after 6 and 12 months of dosing. Tolterodine produced increased heart rate in dogs even at the lowest (0.5 mg/kg) dose which means a no-effect dose has not been identified. A decrease in the PQ-interval was seen in both sexes primarily in dogs receiving 1.5 and 4.5 mg/kg/day. The QRS-interval was unchanged. Changes in the QT-interval probably reflect the increase in heart rate although there was an increase in the QTc interval as well. Drug blood levels (AUC) in these dogs were approximately 10 to 150 times higher than drug blood levels in people taking 2 mg bid. There were unexplained deaths in the mouse carcinogenicity studies at the highest dose. A telemetry study in mice using high doses did not reveal any cardiovascular effects. Doses in the mouse carcinogenicity study produced drug blood levels approximately 10 times higher that the drug blood levels in humans taking 2 mg bid. Free drug blood levels were approximately 45 times higher in mice than in humans.

Little or no target organ toxicity (other than the pharmacodynamic effects noted above) were produced by tolterodine. Some mild liver changes were seen in rats and dogs and increased incidence and severity of alveolar macrophages were seen in the lungs of rats.

Tolterodine has some structural similarities to terodiline which was withdrawn from the market due to suspected associations with cardiac events, particularly Torsades de Pointes and QTc prolongation. Tolterodine does prolong the QTc in dogs and the sponsor was asked to do a 52 week study in dogs to examine the effect of tolterodine on the ECG. Tolterodine increased heart rate in dogs sporadically even at the lowest dose so no no effect dose was identified. A decrease in the PQ-interval was seen in both sexes primarily after treatment with 1.5 and 4.5 mg/kg (drug blood AUC's approximately 10 to 150 times higher than drug blood AUC's in humans taking 2 mg bid). This safety margin decreases to a minimum of 7.6 if tolterodine and DD 01 (the major active metabolite) are combined comparing humans on 2 mg bid vs female dogs receiving 1.5 mg/kg. The minimum margin of safety decreases to 4.2 if free tolterodine plus free DD 01 are compared between humans and dogs. Furthermore, the no effect dose of 0.5 mg/kg produces minimum drug multiples of 3.7 for total tolterodine, 2.6 for tolterodine plus DD 01 and 1.3 for free tolterodine plus free DD 01. Thus the drug exposure level that produced an increased PQ interval may be only slightly greater than the average drug exposure in humans taking 2 mg bid. These multiples of the human exposure are for extensive metabolizers of tolterodine. For poor metabolizers, the drug blood levels will be well above the drug blood levels that cause changes in ECG in dogs. For example, in clinical study 95-OATA-030, when given to extensive metabolizers, 2 mg bid tolterodine gave serum AUC values for tolterodine and DD01 of 62 ug.h/L. In the same study, 2 mg bid given to poor metabolizers resulted in no serum DD01 but an AUC of 216 ug.h/L for tolterodine. Thus the total pharmacologically active moieties are 3.5 times higher in poor metabolizers than in extensive metabolizers and the safety margin is lower by a similar amount.

In the pharmacology safety studies, an iv dose of 0.06 mg/kg to anesthetized dogs (serum levels of 45-87 ng/ml) produced a 6-8% increase in QT, QTc intervals and T-wave amplitude and duration. Doses producing serum tolterodine levels of 398-1510 ng/ml produced a 30% increase

in QT interval, 20% increase in the QTc interval and a 40% increase in the T-wave duration. Following oral administration, there was no change in ECG until serum tolterodine levels reached over 600 ng/ml (increase in the QT interval of approximately 10-20%). In anesthetized cats, tolterodine decreased the T-wave amplitude at serum concentrations of 188 ng/ml and above. The Cmax in humans taking 2 mg bid is approximately 3.6 ng/ml tolterodine.

There were unexplained deaths in the mouse carcinogenicity study at the high dose of 30 mg/kg/day. These doses resulted in drug blood level multiples of 10.4 for tolterodine, 8.9 for tolterodine plus DD 01 and 17.6 for free tolterodine plus free DD 01. For the next lower dose ( the dose that had no increase in mortality) the drug blood level multiples were 3.7 for tolterodine, 3.6 for tolterodine plus DD 01 and 7.8 for free tolterodine plus free DD 01.

If cardiac toxicity's are the result of acute high blood levels of drug (Cmax) then the margin of safety is greater for dogs (26 for tolterodine, 18 for tolterodine plus DD 01 and 8.5 for free tolterodine plus free DD 01). However, for mice, the margin of safety decreases to 11 for tolterodine, 2.2 for tolterodine plus DD 01 and 4.5 for free tolterodine plus free DD 01.

Based on the drug blood multiples between animals and humans, there is not much margin of safety for cardiovascular events. For slow metabolizers of tolterodine, there probably is no margin of safety. Because tolterodine has structural similarities to a previous drug that was withdrawn from the market for possible cardiac effects such as Torsades de Pointes and QTc prolongation, and because tolterodine has similar cardiac effects in animals at fairly low doses, the risk to benefit ratio for tolterodine would seem to be small. However, tolterodine is much more specific for the muscarinic receptor than is terodiline which cross reacts with histamine and alpha-adrenergic receptors and calcium channels. Furthermore, the serum levels of tolterodine at a therapeutic dose are significantly lower than the serum levels of terodiline so the overall safety margin would seem to be much greater for tolterodine than for terodiline.

Electrophysiology studies in isolated cardiac tissue also demonstrated an adequate safety margin for tolterodine. Significant effects on cardiac calcium and sodium channels occurred at concentrations well above those seen in humans taking 2 mg bid. However, this margin of safety will be reduced by approximately one-third in poor metabolizers.

In the rat carcinogenicity study there was a significant increase ($p<0.025$) in malignant renal liposarcoma in males only. The actual numbers were 0/60 control 1, 0/60 control 2, 1/60 LD, 1/60 MD and 3/60 HD. This is a rare tumor with the historical incidence in the lab that did the study ranging from 0 to 1.6% (although the lab has done only a small number of carcinogenicity studies). The 5% incidence in the HD is above the historical control value but the effect was not seen in female rats or male or female mice. Because tolterodine was negative in a large battery of genotoxicity tests the mechanism of carcinogenicity (if the effect is real) is probably epigenetic in nature. The high dose in the rat carcinogenicity study of 30 mg/kg in males results in drug blood levels of tolterodine approximately 13.6 time higher than the drug blood levels in humans taking 2 mg bid. This margin of safety decreases to 7.5 for tolterodine plus DD 01 and to 9.4 for free tolterodine plus free DD 01. However, it is possible that the carcinogenic dose is only slightly above the mid-dose group. In that case the multiples will be significantly lower.

The negative genotoxicity, the weak carcinogenic signal (one sex, one species) and the approximately 10 fold higher drug blood levels in male rats than in humans provides sufficient evidence to consider tolterodine as extremely unlikely to produce cancer in humans. Again, slow metabolizers of tolterodine could be at a higher risk.

There was no effect on fertility (percent of females pregnant) in mice given 30 (M) or 20 (F) mg/kg tolterodine, but there was a reduced number of corpora lutea resulting in fewer implantation sites and fewer viable young. There was also an increased number of early and late resorptions in

these mice. Teratology studies with tolterodine provided evidence that drug treatment can increase the incidence of malformations. In mice there was an increase in cleft palate, digital abnormalities, intra-abdominal hemorrhage and various minor skeletal variations, primarily reduced ossification. In rabbits, tolterodine treatment produced an increase in gross/visceral abnormalities due primarily to an increase in anomalous cervico-thoracic arteries.

## RECOMMENDATIONS

Internal comments: From the standpoint of Pharmacology, Detrusitol can be approved for the treatment of patients with overactive bladder.

External recommendations (to sponsor): None

NDA issues: None

Labeling review: Some minor modifications of the label may be necessary.

Investigator's brochure/Informed consent review:

Reviewer signature/Team leader signature: /S/ 12/22/97

CC list: HFD-580

Appendix:

Draft date:

APPEARS THIS WAY
ON ORIGINAL