MULTIPLE DOSE STUDIES RAT

1. A 28-DAY ORAL TOXICITY STUDY OF RENASTAT IN RATS

Study Nr. GT-01-TX-1. Test facility _____ June 1994. GLP statement included. Lot nr. 3U-187-001. (Vol. 1.13)

Note: Test substance manufactured by block gel process (in contrast to chemical dispersion process used in 1-month rat study GT-01-TX-09; see below).

Methods

Harlan Sprague Dawley (Hsd) rats (10/sex/group), age 5 weeks, were dosed orally (in the diet) for 1 month with **0, 1, 4.5, 10 g/kg/day**. Control group received 10 g/kg cellulose.

Results

Mortality - 1HDm died on Day28. Cause multiple hemorrhages

Clinical signs - pale eyes/skin, ear discharge, alopecia, sores, few feces, thin. swollen testes in HDm

BW - BW gain decreased in HD m,f from Week 1, and in MD from Week 3

FC - No dose-related effects

Hematology - Decrease of RBC, Hb, Hc, MCV in HDm. Increase of WBC, platelet ct. segm neutrophils in HDm

Clinical chemistry - Decrease in HDm,f of albumin, total protein (m only), vit E and vit D, Fe (m only), Zn. Increase in HDm of Cl, P (MD too), ALT (MD too), AST, gluc. BUN, trig, Cu. Increase in ALP in MD,HDf

Vit E: -35% (MDm), -50% (HDm), -50% (HDf)

Vit D: -40% (MDm), -80% (HDm), -50% (HDf)

Fe: -80% (HDm), no effect in f

Urinalysis - no significant changes

Organ weights -

Adrenal, brain, heart, spleen , testes and epididymes: rel wt increased in HDm

Gross pathology - (n=9/group) Findings in HD group:

Skin: crust neck, abrasion 2/9

Lymph nodes: Red 2/9

Epididymis: hemorrhage 4/9, lesion/tan 4/9

Testis: intra-abdominal 2/9, hemorrhage 2/9

Fat (peri-epididymal): hemorrhage 5/9

Carcass: thin 2/9

Liver: pale 2/9

Histopathology -

All findings in HDm:

Hemorrhage in meninges, thymus, muscle, liver, lung, testes, epididymes, fat

Pancreas: inflammation in some animals

Thymus: necrosis in some animals

Liver and spleen: hematopoietic cell proliferation (response to anemia)

Testis/epididymes: atrophy, degeneration, edema, inflammation, oligospermia, necrosis

Fat: inflammation

Conclusion

NOAEL 1 g/kg/day

2. ONE-MONTH REPEATED DOSE TOXICITY STUDY OF PB-94 IN RATS

Study Nr. TX95-125. Test facility/Sponsor [redacted]. Sept 1995-Oct 1996. GLP statement included. Lot nr. 03158ZW00[redacted] (Vols.1.14-1.15)

Methods

Slc:SD rats (15/sex/group), age 6 weeks, were dosed orally (diet) for 1 month with **0, 0, 0.3, 1, 3, 10 g/kg/day**. Second control group received 10 g/kg cellulose. Control was done because 10 g/kg PB-94 is >5% diet content. Dietary route chosen because gavage limit is 2g/kg PB-94, and because of clinical dose administration time (with meals). Two groups (5/sex/group) of 3, 10 g/kg/day were assigned to recovery for 4 weeks.

Results

Clinical signs - Hemophtalmia and enlargement of eye in 3/15 HDm from Day7 or 16. Black feces in HMD, HD (m,f) drug groups, or gray-white feces (cellulose group). Large feces in HMD m,f and HDm. Both findings reversible.

Body weight - Reduced in HD from Day 3 (as compared to control). After that no further change as compared to control. Reduction not seen with cellulose. Finding reversible.

Food consumption - Reduced in HD on Day 3, after that increased in HD [redacted]. Also sporadic increases in other drug groups. FC increased in cellulose group from Day 3. Finding reversible.

EFU - Decreased in HMD, HD as compared to control and cellulose groups.

Actual drug dose levels - In all groups, mean value was 5-15% higher than planned.

Water consumption - Increased in HD as compared to control and cellulose. No effect in cellulose group.

Hematology -

Small decrease in RBC, Hc in HMDm, HDm.

Minimal increase in MCH, MCHC in HMDm, HDm.

(Above changes also seen in cellulose groups, but less pronounced. Females not different from cellulose groups)

Increase in lymphocytes in HMDf, HDf.

Increase in aPTT in HDm,f.

Increase in PT in HDm.

All findings reversible

Ophthalmology - Hemorrhage and enlargement in anterior eye in HDm. Finding partially reversible, but mydriasis (pupil dilation) and lens opacity remained.

Blood chemistry -

HDm,f:

Increase in GPT (HDm 1.5x), Alk Phos (HDm 1.5x), Fe (2x in HDm).

Decrease in total protein, inorganic P (HDm 0.75x), K^+ (HDm 0.75x) and vitamin E (HDm 0.2x).

HDm:

Increase in CPK activity, Ca and Cl (HDm 1.06x, both), A/G ratio, albumin%, gamma-globulin%.
Decrease in triglicerides, bilirubin, a(1+2)-globulin%.

HDf:

Increase in phospholipid, albumin..

HMDm:

Increase in Fe, Cl.
Decrease in triglycerides, and in vitE

HMDf:

Increase in phospholipids
Decrease in ChE.
Note: All changes reversible.

Data on Fe, VitE, VitK at 3 week time point (males)

MALES			
	Fe (ug/ml)	VitE	VitK
control	160	5.8	4.8
LD	170	5.5	4.7
LMD	174	5.1	4.5
HMD	242	3.9↓	4.6
HD	321	1.1↓	3.8↓

Urinalysis -

Increase in volume and decrease in specific gravity in HD.
Increase in Na, K excretion in HDm.
Increase in Ca excretion in HMD, HD (13x, 25x control!!).
Decrease in P excretion (HD 0.01!), and increase in Cl excretion (HD 6x) in LMD, HMD, HD.
Decrease in pH in HD.

Organ weights -

Liver (abs + rel) decreased in HDm.
Brain (rel) increased in HDm,f
Adrenal (rel) increased in HDm

Gross pathology -

Eye, prostate, jejunum, ileum: Dark red foci in in 1/10 or 2/10 HDm

Histopathology -

End of drug treatment period:
Eye: Hemorrhage in ciliary body in 2/10 HDm.
Prostate: Hemorrhage in 2/10 HDm
Jejunum: Congestion in 1/10 HDm.
Ileum: Erosion in 1/10 HDm.
End of recovery period:
Stomach: Calcification in mucosa in 4/5 HDm.
Other histopathology findings not seen or reversed.

Conclusions

Large size feces possibly due to swollen PB-94 compound.

Increase in food consumption in both cellulose and PB-94 groups suggests non-specific effect of polymeric compounds.
Decrease in body weight and EFU related to treatment with PB-94
Increased serum and urinary Cl, and increased urine Na and K may be due to increased intake of Cl present in drug compound; decreased serum and urinary P due to P-binding of PB-94.
Increased serum and urinary Ca is thought to be result of increased calcitriol production due to decreased serum P.
Decrease in RBC and Hc in males maybe due to hypophosphatemia causing reduced erythrocyte ATP.
Prolonged aPTT/PT in HD is probably cause of hemorrhagic events in eye and prostate, and of erosion in GI tract in m. Prolonged blood coagulation time most likely related to decrease in vitamin K absorption and levels. Other bile acid binders have also been shown to reduce vit K absorption and levels.
Decreased serum vit E and triglyceride levels probably due to reduced lipid and lipid-soluble vitamin absorption (also seen with other bile acid binders). However, phospholipid levels in f were increased.
Mechanism of changes in other clinical chemistry parameters unclear.
Decreased liver weight in m may be related to decreased FC and EFU.
Stomach calcification at end of recovery in most HDm possibly due to CaxP increase after drug withdrawal.
NOAEL 1 g/kg/day

3. A 4-WEEK DIETARY TOXICITY STUDY WITH RENAGEL IN RATS

Study Nr. GT-01-TX-9. Test facility _____. January-February 1996. GLP statement included. Lot nrs. 39832-210 and RG-000-0004HRB. (Vols. 1.16-1.17)

Note: Test material manufactured using chemical dispersion process (not block gel process), with 640 ppm residual isopropranol.

Methods

Crl:CD(SD)BR VAF/plus rats obtained from _____ (10/sex/group), age 5 weeks, were dosed orally (in the diet) for 4 weeks with **0, 1, 4.5, 10 g/kg/day**. The control group received 10 g/kg cellulose.

Results

Mortality - 2/10HDm died and 6/10 HDm were sacrificed before study termination. Cause probably related to multiple hemorrhages in these animals.
Clinical signs - Paleness, red discharge from eyes, genitals, nose, mouth in HDm. Discharge from eye, nose also in MDm.
BW - BW gain decreased in HDm
FC - FC decreased in HDm
Ophthalmology - Hemorrhage and retinal bleeding in 1/10MDm and 1/10HDm
Hematology -

Findings in HDm that were sacrificed moribund:
Anemia (decreased RBC, Hb, Hc)

Markedly increased PT and aPTT
Increased neutrophil ct, increased reticulocyte count and decreased lymphocyte ct
In other MDm, HDm and HDf
Increased aPTT and decreased MCV

Clinical chemistry -

Findings in HDm:
Increased glucose, triglycerides, alpha-1 globulin, and decrease in serum Ca.
Decrease in Fe in HDf, not in HDm.
Findings in MDm,f and HD m,f:
Increase in BUN (m>f), cholesterol, ALT, AST, ALP (m>f)
Decrease in albumin, total protein, serum Cl,, serum K (m>f)

		Vit A	Vit D	Vit E
males	control	0.36	24.4	4.5
	LD	0.40	23.2	4.4
	MD	0.41	7.9*	2.2*
	HD	0.095*	-	1.7*
females	control	0.17	27.3	7.9
	LD	0.17	28.5	6.1
	MD	0.19	16.4*	4.1*
	HD	0.24	6.01*	3.2*

Urinalysis -

In MD, HD:
Increase in specific gravity, osmolality, Cl excretion.
Decrease in K excretion.

Organ weights -

Pituitary: decrease (abs in m,f, and rel in f) in HDm,f
Heart: Increase (rel) in HD m,f
Adrenal: Increase (rel) in HDm
Testes: Increase (rel) in HDm
Brain: Increase (rel) in HDm
Ovary: Increase (rel) in LD, MD, HDf

Gross pathology -

Findings in HDm that died/were sacrificed (n=8):
Red lesions in epididymes, muscle, thymus, and lymph node in 3-6 animals, and occasionally in pancreas, prostate, penis, seminal vesicles, thyroid, lymph nodes, indicating hemorrhage

Findings in surviving HDm:
Enlarged, red epididymis, large testis, red thymus and red lymph nodes in 1/2

Histopathology -

Findings in unscheduled necropsied HDm (total n=8):
Epididymes: Inflammation 5, hemorrhage 7, hypospermia 3
Testis: Atrophy 2
Thymus: Hemorrhage 8, ischemic necrosis 4
Prostate: Hemorrhage and necrosis 1

Brain: Hemorrhage 2

Spleen: Diffuse reticuloendothelial hyperplasia in red pulp in 2/8 HDm, and in 2/10 LDm.

Femur, sternebrae: Physeal dysplasia (widened cartilage zone in growth plate, and decreased osteoid deposition in primary spongiosa.

Findings in scheduled necropsies (10-10-10-2):

Epididymis hemorrhage and inflammation, thymus hemorrhage and infarction, enlarged testis with atrophy in 1 HDm.

Femur, sternebrae physeal dysplasia in 2HDm.

Femur, sternebrae physeal dysplasia in MDm (5/10)

Conclusions

Majority of HD animals died with multiple tissue hemorrhages. There was no vascular injury observed in hemorrhaging tissues. Hemorrhage possibly due to Vit K deficiency. However, Vit K levels not measured.

Vit A,E, D levels all decreased in MD and HD, or HD animals.

Physeal dysplasia seen in MD and HD males indicates impairment of ossification possibly due to vit D deficiency.

Spleen finding in HDm that died indicates immunologic stimulation/systemic stress.

NOAEL 1 g/kg/day

4. 28-DAY ORAL TOXICITY IN HARLAN SPRAGUE DAWLEY (HSD) RATS AND CHARLES RIVER SPRAGUE-DAWLEY (CD) MALE RATS (From Special Toxicity section)

Study nr. GT-01-TX-21. Test facility _____. December 1996-January 1997. GLP statement included. Lot nrs. Renagel 18323NI00, Cholestagel TK FC402-1498. (Vol. 1.27)

Purpose

Assess toxicity of Cholestagel and Renagel administered to (SD rats of) two different vendors.

Methods

Male Sprague Dawley (SD) rats were purchased from Harlan Sprague Dawley (Hsd rats) or Charles River (CD rats). Rats (10/group), age 7-9 weeks, were dosed orally (in the diet) for 28 days with **0, 3.6 g/kg/day Cholestagel, or 10 g/kg/day Renagel**. The control group received no test article.

Results

Mortality -

CD; Cholestagel	1/10 died (Day 22), 1/10 Renagel (Day 11)
Hsd; Cholestagel	3/10 died (Day 24, Day 25, killed moribund Day 27)

Clinical signs -

CD; Cholestagel	red staining of ears and paws, hair loss
Hsd; Cholestagel	red eye staining
CD; Renagel:	red staining, paleness, hair loss, decreased activity, coldness in 1/10
Hsd; Renagel	No findings

BW -

CD; Cholestagel	BW and/or BWG reduced D14-27
Hsd; Cholestagel	BWG reduced D27
CD; Renagel	BW and/or BWG reduced D7-27
Hsd; Renagel	BW and/or BWG reduced D7,27

Generally, BW (gain) reduction in CD rats larger than in Hsd rats

FC -

CD; Cholestagel	FC decreased D27
Hsd; Cholestagel	FC increased D7-27
CD; Renagel	FC increased D14
Hsd; Renagel	FC increased D7-27

Ophthalmology -

CD; controls	striate retinopathy in 1/10
CD; Cholestagel	pale fundi in both eyes of 2/10
Hsd; Cholestagel	striate retinopathy
CD; Renagel	vitreal hemorrhage in 1/10
Hsd; Renagel	No findings

Hematology -

CD; Cholestagel	Decrease in RBC, Hb, Hct. Increase in platelet and reticulocyte values. Increase in neutrophil values. Increase in PT and aPTT.
Hsd; Cholestagel:	Increase in platelet count. Increase in PT and aPTT.
CD; Renagel:	Increase in reticulocyte counts. 1/10 anemic. Increase in PT and aPTT.
Hsd; Renagel:	Increase in platelet count. Increase in aPTT.

Clinical chemistry -

CD; Cholestagel	Albumin decreased, globulin increased, A/G ratio decreased. Decrease in protein values. Decrease in Vitamin E.
Hsd; Cholestagel	Albumin decreases, globulin increased, A/G ratio decreased. Decrease in protein values. Decrease in Vitamin E.
CD; Renagel	Decrease in Vitamin E. Increase in ALP.
Hsd; Renagel	Decrease in Vitamin E. Increase in ALP.

Urinalysis -

No treatment-related findings.

Organ weights -

CD; Cholestagel	Body decreased 20%; Liver abs decreased; Spleen abs/rel increased; Kidney abs decreased; Adrenals rel increased; Brain rel increased
Hsd; Cholestagel	No effects
CD; Renagel	Body decreased 20%; Heart abs decreased; Liver abs decreased; Adrenal rel increased; Brain rel increased
Hsd; Renagel	Adrenal abs/rel increased

Gross pathology -

CD; Cholestagel	Pericardial fat white/dark foci, discoloration; Liver pale; Spleen enlarged; Lungs firm with dark apical lobes; Prostate hemorrhage;

	Epididymes hemorrhage; Testes soft with hemorrhagic/pale seminal vesicles.
Hsd; Cholestagel	Testes: Hemorrhagic inguinal canal.
CD; Renagel	Heart dark discoloration and firm; Testes (r) flabby with hemorrhagic/pale seminal vesicles
Hsd; Renagel	No treatment-related findings
Histopathology -	
CD; Cholestagel	1 animal that died: severe liver damage. Other animals: Spleen increased severity of extramedullary hematopoiesis; Heart epicarditis 4/10; Liver increased severity of mononuclear cells and nunsuppurative hepatitis, hepatocellular degeneration 2/10; Lung/Thymus mod/severe inflammation and hemorrhage 2/10; Skeletal muscle hemorrhage 2/10; Epididymes inflammation 5/10.
Hsd; Cholestagel	2 animals that died: 1 cystitis, 1 perirenal hemorrhage with cheese. 1 animal that was killed: double cystitis. Other animals: Epididymes inflammation 5/10. Urinary bladder acute inflammation 2/10.
CD; Renagel	Heart epicarditis 2/10; Liver increased severity of mononuclear cells and nunsuppurative hepatitis; Epididymes inflammation 1/10; Lung/Thymus moderate to severe inflammation and hemorrhage 2/10;
Hsd; Renagel	No treatment-related findings

Conclusions

Vitamin E deficiency in both vendors, by both Cholestagel and Renagel. CD rats more susceptible with respect to BW, signs, hematology, ophthalmology, gross and microscopic pathology, organ weights to both Cholestagel and Renagel.

A 12-WEEK ORAL TOXICITY RANGE-FINDING STUDY OF RENAGEL IN RATS

Study Nr. GT-01-TX-5. Test facility Geltex. December 1994. Lot nr. 14-153. GLP statement provided.

Methods

SD rats (4/sex/dose group), 7 wks old, were dosed orally, in the diet, for 12 weeks with **0, 0.6, 2, 6 g/kg/day**. Control group received 6 g/kg/day of cellulose. Actual HD level was 7g/kg/day. HD diet consisted of 8-10% Renagel. Signs, BW, FC were monitored, and necropsy was done on all animals.

Results

No signs

No effects on BW

FC variable: Increased FC in HDm,f.

No gross lesions

Conclusion

NOAEL >7 g/kg/day

5. 90-DAY ORAL (DIET) TOXICITY STUDY OF RENAGEL IN RATS

Study Nr. GT-01-TX-16. Test facility_____ December 1996-March 1997. Lot nrs. 17302NI00, 13224NI00, 18323NI00. GLP statement provided.

Methods

Hsd: SD rats (15/sex/dose group), 7 wks old, were dosed orally, in the diet, for 90 days with **0, 1, 2, 4.5, 10 g/kg/day**. Control group received 10 g/kg/day of cellulose. Diet was available ad libitum. After 28 days, 5/sex/group were killed and necropsied. The other 10/sex/group continued treatment through 90 days.

Results

Mortality - Two HDm found dead on Days 54 and 58, one without previous signs, other with signs of hemorrhage from nose and rectum. One HDm killed in extremis on Day 88, with signs of hemorrhage. Deaths considered test article related.

Clinical signs - Chromodacryorrhea in 1LDm, and 1 LMDm.

BW - Decrease in BW in HDm (ca. 0.85x at 90 days), and HDf (0.9x at 90days), mainly due to decreased BW gain in first 3 wks of study.

FC - Variable: Decreased in LD, LMD and increased in HD, m and f.

Test article intake - Actual dose within 1% of intended dose

Ophthalmology - No treatment-related findings

Hematology - Week 13: RBC decreased in HMD, HD m and f. Platelet count increased in HMDm, HDm, HDf. MCH increased in HMDm, HDm, HDf.

Clinical chemistry -

Week 13:

Serum Na, Ca (f) slightly increased, serum K and Cl slightly decreased in HD. P increased in HMD, HD m and f (up to 30%)

ALT slightly increased in LMD, HMD, HD.

AST slightly increased in HDm, and in LMD, HMD, HDf.

BUN increased (1.5x) in HDm,f.

Vit E: Decreased dd in all male groups; decreased in HMDf, HDf (HDm 0.2x, HDf 0.3x)

VitD: Decreased in HMDm, HDm; decreased in HDf (HDm 0.15x, HDf 0.5x)

Urinalysis - No data

Organ weights -

Brain: Increased (rel) in HDm

Kidneys: Increased (rel) in HDm

Testes/epididymes: Increased (rel) in HDm

Liver: Increased (abs and rel) in LMD, HMD, HDf

Kidneys: Increased (abs and rel) in HMD, HDf

Gross pathology -

28 day sacrifice

No findings

90 day sacrifice

Hemorrhage in various internal organs in 3 animals that died prematurely

Histopathology -

28 day sacrifice (N = 5/group)

No treatment-related findings
90 day sacrifice (N=10/group)
Spinal cord: Hemorrhage and vacuolation in white matter 1HDm (in moribund sacrifice). Meningeal hemorrhage 1HDm (in animal found dead)
Lungs: Vascular mineralization/hypertrophy 1HDm
Kidneys: Mineralization, tubules 2HDf , 1 control m, 1HDm
Liver: Necrosis hepatocytes 1HDm
Pancreas: Fibrosis in 1HDm
Spleen: Lymphoid hyperplasia 1HDm, lymphoid depletion 1HDm
Stomach: Edema, submucosa in 1HDm, 1LDf, 2HDf
Colon: Lymhoid hyperplasia, Peyer's patches 1HMDf, 1HDf
Ileum: Lymhoid hyperplasia, Peyer's patches 1LDm, 1HDm, 2LMDf. 3HDf
Rectum: Edema, submucosa in 1HMDm, 1HDm, 1LMDf
Thymus: Hemorrhage in **1LDm**, 2HDm. Atrophy in 1 HDm.
Skeletal muscle: Hemorrhage in 1 HMDm
Urinary bladder: Dilated lumen 3HDm
Uterus: Dilated lumen: 1control f, 1LDf, 2HMDf, 4HDf
Prostate: Atrophy 1HDm.
Eyes: Hemorrhage 1HMDm, 1HDm

6. A 13- AND 26-WEEK ORAL (DIET) TOXICITY STUDY OF RENAGEL IN RATS

Study nr. GT-01-TX-6. _____ Lot nr. RS9401HR and RS9502HRC.

Methods

Hsd SD rats (20/sex/group), 6 weeks old, were treated orally, in the diet, with **0 (cellulose 6 g/kg/day), 0.6, 3.0, 6.0 g/kg/day**. Fresh diets presented weekly. 10/sex/group killed after 3 months, 10/sex/grop killed after 6 months.

Results

Mortality - In 3-mo study, one control died due to anesthetic overdose. In 6-mo stud, 3 deaths: 1 control m with adenocarcinoma in Zymbal's gland, 1 LDm, 1 HDm (Day 100: languid, hypothermic, pale, hemorrhage in abdominal cavity)
Clinical signs - considered incidental
BW - BW reduced in HDm in first 13 weeks, BW gain slightly decreased over 6 months. No effect in females.
FC - Reduced in LD f in week 1-13.
Hematology - Week 7: PT and APTT values "suspect; not reported"
Week 13: WBC increased in HDf. APTT increased in HDm (not sign) and HDf (sign)
Clinical chemistry - *(assays at wks 7,13,26)*
Serum P increased in males MD, HD (all times), and in females MD. HD (wk 7,13)
Serum Cl minimally but sign decreased in HDm,f (all times)
ALT increased in m and f MD,HD (up to 2x in wk26) (all times)
AST increased in m and f HD (up to 1.5x) (wk 13,26)
BUN increased in m and f HD (up to 1.5x) (wk 13,26)

Cholesterol slightly increased in various treated (wks7,13), decreased in HDm (wk 26)
Albumin slightly decreased in MD,HDm (wk 13, 26) and MD,HDf (wk 13)
Vit A unchanged
Vit E decreased in m LD,MD,HD (down to 0.25x) and f MD,HD (0.5x)
Vit D decreased in HDm (0.3x) at wk 13), but unchanged at wk 26
Cu increased in MD, HDf (wk 13, 26); increased in HDm only in wk13
Fe decreased in LD,MD,HDm (wk 13), and HDf (wk 13, 26)

Urinalysis - no data

Organ weights -
Wk 13: no drug-related changes
Wk 26: no drug-related changes?
Kidney: increased (abs/rel?, unclear) in HDm and in MD,HDf

Gross pathology - changes concidered incidental

Histopathology -
Lung: Crystallin material in HDm that died
Stomach: Edema, submucosa in females LD,MD,HD at both times, and slightly increased incidence in males LD,MD,HD at wk26
Intestine: Crystalloid material in lumen in HD
Thymus: Hemorrhage 2/10 HDf wk13; but also in controls and HD wk26
Pancreas: Acinar cell degeneration in 1/10 HDf wk13
Eye: Retinal degeneration in 1/10 HDf wk 26
Optic nerve: Malacia in 1/10 HDf wk 26
Liver: Necrosis in 1/10 HDf wk 26

MULTIPLE DOSE STUDIES DOGS

1. TWENTY-EIGHT DAY SAFETY AND TOXICITY STUDY OF THE TEST ARTICES ADMINISTERED IN THE FEED TO DOGS

Study Nr. GT-01-TX-3. Test facility _____ December 1993. GLP statement included. Lot nr. 17-012

Methods

Female Beagle dogs (4/dose group), 2-4 years old, were dosed orally (in feed) for 28 days with **0, 1.2, 4, 12 g/day, ie approximately 0, 0.12, 0.4, 1.2 g/kg/day**. Control group received 12 g/day of methyl cellulose. Feed was high fat canned diet. Necropsy was done on 2 animals from control and HD group.

Results

Mortality, signs, BW, FC, ophthalmology - no drug effects
Hematology - RBC, Hb, Hc ca 20% decreased from Day 15 in HD
Clinical chemistry -
Serum Ca decreased in HD
Serum Cl increased in MD, HD
Gross pathology -
Spleen: white foci (local capsular fibrosis) in HD

Conclusion

NOAEL 0.4 g/kg/day

2. A 28-DAY ORAL (CAPSULE) TOXICITY STUDY OF RENAGEL IN THE BEAGLE DOG

Study Nr. GT-01-TX-2. Test facility _____. June 1994. Lot nr. 3U-187-002. GLP statement provided.

Methods

Beagle dogs (4/sex/dose group), 5-7 months old, ca. 9.5kg at study initiation, were dosed orally, with a capsule, for 28 days with **0, 0.2, 1, 2 g/kg/day**. Feeding was done during a 1-h daily feeding period in first 3 study days. From Day 3 dogs were fed overnight (21h), then fasted for 2h prior to dosing. Unclear when feeding was resumed post dosing. Control group received 2 g/kg/day of methyl cellulose.

Results

Mortality - None
Clinical signs - Yellow gel-like mass in feces (3/8MD, 5/8HD), red gel-like mass in feces (1/8 control, 2/4LDf), red-yellow particles in feces (2/4MDf, 4/8HD)
BW - Slight decrease in BW, and actual BW loss in 2 animals in HDm
FC - decreased sporadically in HDm and HDf
Ophthalmology - No effects
EKG - All "within normal limits"
Hematology - No effects on RBC, Hb, HC. Decreased platelet count in HDm and HDf, significant in f in Week 2 and 4

Clinical chemistry -

	cholesterol	trig	Cl	Fe	VitD	VitE	ALT
males	-40% HD, dd	-40% HD	+6% HD, dd	-50% HD, dd	+40%, LD, MD; -30% HD	-40% HD	557 in 1 HDm with BW loss (vs. 35 control)
females	-40% HD, dd	-50% HD	+3% HD	-40% HD, dd	+40%, LD, MD; -30% HD	-50% HD	

Urinalysis - Decrease in urobilinigen in MD, HD m and in HDf.
Organ weights - No effects (abs or rel)
Gross pathology -
Epididymis: Small in 1/4 HDm
Gall bladder: Thickened 1/4 HDm
Stomach: Thickened 1 HDm
Colon: Dark area 1HDf
Brain: Dilatation 1HDf
Testis: Small 1 HDm
Histopathology - (control and HD examined only)
Brain: Infiltration, mononuclear cell 1HDf. Dilatation ventricular 1 HDf
Bone marrow: increased hematopoiesis 1HDf
Gallbladder: Mucosal hyperplasia 1HDm
Esophagus: Erosion 1HDm
Mesenteric lymph node: Hemorrhage 2 HDm, 1 control f, 1HDf
Thymus: Lymphoid atrophy 1HDm

3. ONE MONTH REPEATED DOSE TOXICITY STUDY OF PB-94 IN DOGS

Study Nr. CH95147. Test facility _____. October-December 1995. Lot nr. 03158ZW00. GLP statement provided.

Methods

Beagle dogs (5/sex/dose group), 7 months old, ca. 8-10kg at study initiation, were dosed orally, with a capsule, for 28 days with **0, 0.2, 0.6, 2 g/kg/day**. Dose was administered between 10 am and 12 pm. Food was provided daily, not mentioned at what time. Control group received empty capsules. 2 animals from control, MD, HD were used for 4 week recovery period.

Results

Mortality - None
Clinical signs - Yellow stool in 3/5 HDm and 5/5 HDf. Occasional vomiting in HDm,f.
BW - Decrease in BW gain in MDm, HDm and HDf
FC - No effects
Water consumption - Increased in HDm,f throughout treatment.
Ophthalmology - No effects
Hematology - MCH decreased and PT increased in HDm in Week3.

Clinical chemistry -
Increase in serum Cl in HDm,f
Decrease in total and free cholesterol, phospholipid and VitE in HDm.
Urinalysis - Increased Cl excretion in MD, HD m,f. Increased Ca excretion in HDm,f.
Organ weights - Decrease in spleen (abs and rel) in HDm. Reversed upon recovery.
Gross pathology - No effects
Histopathology - (3/group treatment, 2/group treatment + recovery)
Liver: Hemorrhage and fibrosis near gallbladder in 1/3 HDf.
Gallbladder: Hemorrhage in capsule in 1/3 HDf
Lung: Accumulation of foam cells in alveolar space in 1/3HDm. Bronchiolitis in 1/3HDm.
Prostate: Inflammation in 1/3HDm

Conclusion
NOAEL 0.6 g/kg/day

4. A 13-WEEK ORAL (CAPSULE) TOXICITY STUDY OF RENAGEL IN BEAGLE DOGS

Study Nr. GT-01-TX-7. _____. April-July 1995. Lot nrs. RS9501HRB, RS9501HRD, RS9502HRC (1 lot/month)

Methods
Beagle dogs (4/sex/group) were treated orally, by capsule, **with 0 (10 g/kg/day methylcellulose), 200, 1000, 2000 mg/kg/day**. Dogs were fed overnight and food was removed appr. 1-2h prior to dosing.

Results
Mortality - 1 MDm killed moribund 1 day before necropsy
Clinical signs - No drug effects
BW - No drug effects
FC - No drug effects
Ophthalmology - No drug effects
EKG - No drug effects
Hematology - RBD, Hb, Hct slightly decreased in HDm (wk7), and in HDf (wk14).
Eosinophile ct increased in HDf wk14.
Clinical chemistry -
Cholesterol slightly reduced in HDm (wk7), and MD,HDf (wk7,14)
Triglyc reduced in HDm (wk7)
Serum P slightly increased in MD,HD, both m and f (wk13)
Serum Cl increased in HDm,f (wk7,13)
Vit A unchanged in m or f (wk13)
Vit E decreased in HDm, and in MD,HDf (wk13)
Vit D slightly reduced in HDf (wk13)
Urinalysis -
Ca content increased in MD,HD both m and f (up to 3x) (wk7)
Cl content increased in MD,HD both m and f (up to 2.5x) (wk7,13)
K, Na content decreased in MD,HDf (wk7)

Organ weights - No significant effects
Gross pathology -
Findings in MDm that died:
Dark discoloration of cecum, digesta, rectum, heart
Intussusception in colon
Other animals:
Testis, epididymis: Small (1 HDm)
Lung: Dark area 1HDm, and 1LDf, 2HDf. Pale area 1LDm, 1HDm
Histopathology - (examined control and HD only)
Findings in MDm that died:
Cecum: Multifocal hemorrhage
Heart: Hemorrhage with myocardial degeneration and/or necrosis/inflammation
Pancreas: Decrease of zymogen
Mesenteric lymph node: Congestion
Thymus: Lymphoid necrosis
Other animals:
Lung: Bronchopneumonia 1HDm, 2HDf.

5. A 12-MONTH ORAL (CAPSULE) TOXICITY STUDY OF RENAGEL IN THE BEAGLE DOG

Study Nr. GT-01-TX-10. _____ May 1996- June 1997. Lot nrs. 13223NI00, 18323NI00. GLP statement provided.

Methods

Beagle dogs (6,4,4,6/sex/group), 7-9 months old, 6-11kg, were treated orally, with 4,1,2,4 gelatine capsules, once daily in the morning for one year, **with 0, 200, 600, 2000 mg/kg/day**. Animals were fed 400g of diet each day. 2/sex/group were left untreated for additional 4 weeks.

Results

Mortality - No treatment-related deaths
Clinical signs - Colored feces frequently seen throughout study in HDm,f. Coated feces occasionally seen in HD. Loose or liquid feces, and vomiting occasionally seen in all groups. 1 control was killed after 28 days with tracheobronchitis, and was replaced.
BW - No treatment effects
FC - No treatment effects
WC - Increased 1.5x in HD
Ophthalmology - No treatment effects
EKG - No treatment effects
Hematology -
Week 52:
RBC, Hb, Hct decreased in MD,HDm, and in HDf
MCV increased in HDm,f
MCHC decreased in HDm,f.
Week 56:
After recovery, no differences between control and HD.

Clinical chemistry -

Cholesterol decreased in HDm,f in wk 26, only in HDf in wk52.
Phospholipids decreased in HDf wk26,52
Triglyc decreased in MDm wk52 and in HDm,f wk26,52
Free fatty acids (NEFA) decreased in HDf wk26
ALP increased in HD wk 26,52 (both liver and bone isozymes).
Serum Cl increased in HDf wk26,52, and in HDm wk26
Fe increased in HDm wk26
Vit D (controls wk 52: m 43, f 29) decreased in HDm wk26,52
Vit E (controls wk 52: m 15, f 20) decreased in HDm,f wk26,52
Folic acid decreased in MD,HDm, and in HDf (data only from wk52)
Vit K increased (!) in HDm wk26, and decreased in MDf wk52

Urinalysis - Volume, and total Cl (m 3x), Ca (m 1.5x), P (m 2x) excretion increased in HDm,f, sign in m only

Organ weights -

Liver: Increased (rel) in HDm
Kidney: Increased (rel) in HD

Gross pathology -

Stomach, intestine: Abnormal contents, pale, thickening in 1HDm. Pitted surface in 2 recovery HDm.

Histopathology -

Adrenals: Focal mineralization slight in 1/4 HDm
Gallbladder: Epithelial vacuolation in 1 control m, 2HDm, 1HDf
Kidney: Tubular pigment deposits containing lipofucsin, m 3-2-2-0, f 4-2-1-0. Increased collecting tube vacuolation (lipid containing) in 1HDf. Focal tubular epithelial hypertrophy in 1 HDm.
Spleen: Focal capsular fibrosis in 1HDm, 1HDf
Testes: Interstitial hemorrhage 1MDm
Tonsil: Hemorrhage 1MDf

APPEARS THIS WAY
ON ORIGINAL

REPRODUCTIVE TOXICITY

1. AN ORAL FERTILITY STUDY OF RENAGEL IN THE RAT

Study Nr. GT-01-TX-13. April - June 1996. Lot nr. 13224NI00. GLP statement provided.

Methods

Crl:CD(SD)BR rats (20 sex/group) were treated orally, by dietary admix, **with 0, 0, 0.5, 1.5, 4.5 g/kg/day**. Dose levels based on 4-wk toxicity study. Concentration of test substance in HD diet was 5-6%. One control group received cellulose (Sigmacell) at 4.5 g/kg/day. Males were treated for 28 days before mating, throughout mating, until necropsy. Females were treated for 14 days before mating, through Gestation Day 7 (GD7). Mated females were necro-spied on GD13.

Results

Mortality - 1HDm died in wk7 of study. Animal had dark fluid in trachea, dark colored digesta, and dark discoloration of gastric mucosa and lymph nodes. Death was most likely treatment-related, due to impaired blood coagulation process.

Clinical findings - No treatment effects

Body weight -

BW gain decreased in HD Day 0-24. BW slightly decreased in HDm, minimally decreased in LDm, throughout study.

BW gain decreased in HDf Day0-14. BW slightly decresaed in HDf throughout gestation.

Food consumption -

Males:

Increased in Sigmacell m. Increased in HDm as compared to Sigmacell m. MDm same as Sigmacell m control.

Females:

Premating: Increased in Sigmacell f . MD,HDf same as Sigmacell control.

Gest Day 0-7: Increased in Sigmacell f. Increased in HD f as compared to Sigmacell control. MDf similar as Sigmacell f.

Gross pathology -

Digesta, ingesta: Discoloration in HDm 1,1

Lymph node: Discoloration m 0-0-0-0-2. Enlargement m 0-0-0-0-1.

Lymph node, mandibular: Enlargement m 1-2-3-3-3, f 2-5-3-3-7

Prostate: Small 0-0-0-1-1. Foci/area dark HD 0-0-0-0-1/1.

Stomach: Dilatation HDm 1. Dark area HDm 1

Testis: Area pale HD 1

Trachea: Fluid HDm 1

Organ weight -

Testis, epididymis: Increased (rel), right one, in LD, HD (Bw decreased in these 2 groups)

Histopathology -

Testis, epididymis: No changes

Estrous cycle - Unaffected

Reproductive parameters:

Parental findings:

Mating indices - No effect (All 100%)
Fertility index - No effect (range 70-90%)
Mean day of mating - Decrease (from 3.5 to 2) in LD,MD groups. No effect in HD. Effect probably related to variation in estrous cycle stage.
Conception rate - No effect
Sperm motility (% motile sperm) - No effect (range 77-82%)
Sperm count - No effect (range 840-950.10^6/g)
Sperm morphology (head-flagellum-connection)- No effect

Ovarian/Uterine findings:

Corpora lutea - Not affected (mean # range 14.9-16.2)
Implantation sites - Not affected (# range 14.1-15.5)
Live embryos - Not affected (# range 13.1-14.4)
Dead embryos - No effect (# range 0-0.1)
Early resorptions - No effect (# range 0.6-1.2)
Sum of dead embryos + early resorptions - No effect
Preimplantation loss - No drug effect (3.4%-6.3%)
Postimplantation loss - No drug effect (3.8%-7.8%)
Sum of pre- + postimplantation losses - No effect

Conclusions

Renagel at doses up to 4.5 g/kg/day does not affect male or female rat fertility. Dose selection was adequate.

Comments

Male rats were treated for "at least 28 days" before premating. It is not clear how long the treatment actually was. However, 28 days is sufficient according to new ICH guidelines. Females were treated though Gestation Day 7 ("Japan study design"), and were all killed on Day 13. Thus, parturition and lactation were not allowed to occur, and the F1 was not tested for survival, morphology, growth or fertility.

2. A DIETARY TERATOLOGY STUDY OF RENAGEL IN THE RAT

Study nr. GT-01-TX-14. June-August 1996. Lot nr. 13224NI00. GLP statement provided.

Methods

Female SD rats (25/dose group), naturally inseminated, were treated orally. by diet, with **0, 0, 0.5, 1.5, 4.5 g/kg/day** from GD6-GD17. One control group was given 4.5 g/kg/day Sigmacell. Animals were killed on Gest Day 20, and tissues, ovaries and uterus were examined. Fetuses were examined, weighed, and killed. Abnormalities are classified as:
-major malformations
-minor external and visceral anomalies
-minor skeletal anomalies
-common skeletal variants

Results

Mortality - None
Signs - No treatment-related
BW - No effects
FC - Period Gest Day 9-18: Minimal increase in Sigmacell f as compared to basal diet control. Dose-dependent small increases in LD, MD, HD of up to 10% as compared to Sigmacell control.
Gross pathology -
No drug related findings
Reproductive parameters:
Number mated - No effect (25-25-25-25-25)
Number pregnant - No effect (21-22-23-24-23)
Number dying - None 0-0-0-0-0
Uterine/ovarian findings:
Number of total resorptions - None 0-0-0-0-0
Total number of corpora lutea - Not affected (mean # range 16.8-17.6)
Total number of implantation sites - Not affected (15.2-15.9)
Sex ratio fetuses - Not affected
Dead fetuses - None 0-0-0-0-0
Live fetuses - No effect (14.6-15)
Total number of resorptions - No effect (0.6-1) (No effect on early, middle, or late resorptions)
Preimplantation loss - No effect (5.7-11.9%)
Postimplantation loss - No effect (3.6-7.1%)
Uterine weight - No effect(87-92g)
Fetal weight - No effect (3.8-4.0g)
Incidence of abnormalities:
Major malformations - Fetal incidence 1/325, litter incidence 1/22 in LD, none in other groups, ie, no drug effect
Minor external and visceral anomalies - Fetal (litter) incidence: 2/306(21) - 0/325(22) - 0/346(23) - 2/357(24) - 1/339(23)

Minor skeletal anomalies -

	Total number of fetuses examined 306-325-345-356-339	Total number of litters examined 21-22-23-24-23		
Finding	FA = number of fetuses affected	LA = number of litters affected	FA sign in	LA sign in
Reduced ossification of hyoid bone (skull)	30-36-43-53-89	15-16-15-17-21	HD	
Irregular ossification of supraoccipital bone (skull)	9-5-7-15-17	5-4-5-10-9		
Reduced ossification of sacral verebral arches (vertebral column)	1-1-3-11-20	1-1-3-4-7	MD, HD	
Reduced ossification of ribs	0-1-1-3-4	0-1-1-2-2		
Reduced ossification of ischial bone (pelvic girdle)	4-4-4-10-18	2-3-3-3-9	HD	

Reduced ossification of pubic bone (pelvic girdle)	10-12-21-27-49	4-8-10-9-16	MD, HD	HD

Common skeletal variants -
No effects on
1. thoracic centrum
2. sternebrae 1 to 4
3. sternebrae 5 and xiphisternium

Conclusion -
Treatment of pregnant female rats from Gestation Day 7-19 results in increased fetal and litter incidence of minor skeletal anomalies. The anomalies included reduced and irregular ossification of various bones. Findings indicate reduced rate of fetal bone ossification by Renagel at dose levels of 1.5-4.5 g/kg/day.

3. AN ORAL RANGE-FINDING TERATOLOGY STUDY OF RENAGEL IN THE RABBIT

Study Nr. GT-01-TX-14. _____ October 1995.
Lot nr. R59502HRC. GLP statement provided.

Methods -
New Zealand White rabbits (5/dose group), 4-5 months old, 2.6-3.4 kg, artificially inseminated, were treated orally, via gastric intubation, at same time each day, with **0, 150, 500, 1500 mg/kg/day** on Gest Days 7-19. Controls received only vehicle (water). Animals received 180g Certified _____ per day. On Gest Day 29, animals were killed, and ovaries and uteri were removed and examined.

Results -
Mortality -
Found dead: 2MD (GD11,GD16), 1HD (GD15)
Sacrificed moribund: 2 MD (GD8, GD15)
(The MD sacrificed on Gest Day 8 was replaced)
Sponsor states that deaths were due to intubation errors.
Clinical signs - In 3/5 animals that died or were sacrificed, labored breathing occurred.
Otherwise, no treatment related findings
BW - (data from n = 3-5-3to1-5to4) No effects
FC - (data from n = 3-5-4to1-5to4) No effects
Gross pathology -
Animals that died (4MD, 1HD):
MD: Perforation of esophagus, material in thoracic cavity
MD: Heart raised areas, lung multiple dark areas, subcutaneous tissue multiple dark areas, thymus dark areas, vagina dark material, adhesion.
MD: Lung dark depressed area
MD: Esophagus multiple perforations, material in thoracic cavity
HD: Jejunum dark and/or raised areas, lung multiple dark areas, thymus dark areas
Others:
Lymph node: Enlargement 0-0-0-1

Thymus: Area dark 0-0-2-1. Swelling 0-0-0-1.
Vagina: Material dark 0-0-1-0

Reproductive findings:

Number inseminated 5-5-6-5
Number pregnant 4-5-3-5
Pregnancy rate 80-100-60-100%

Uterine findings (data from n = 3-5-1-4):

Corpora lutea - Not affected (mean # range 9-11.3)
Total number of implantation sites - Not affected (7-9)
Sex ratio fetuses - No significant effect
Dead fetuses - None 0-0-0-0-0
Live fetuses - No effect (5.8-8.4)
Total number of resorptions - No effect (0.6-1.7) (No effect early, middle, or late resorptions)
Number of empty implantation sites - No effect (0-0-0-0)
Preimplantation loss - No significant effect (0-43%)
Postimplantation loss - No significant effect (7-18.1%)
Uterine weight - No effect
Fetal weights - No significant effect

Abnormalities: (Fetuses examined 21-42-8-23; Litters examined 3-5-1-4)

No major malformations
No minor anomalies

Conclusions

Renagel did not cause embryolethality or teratogenicity in the dose range used.
The study showed mortality at doses of 500 and 1500 g/kg/day. The signs and pathology seen in animals that died is consistent with intubation errors as cause of death. However, deaths may also have been related to drug-induced toxicity. Although there was no clear drug-related pathology in the animals that did not die, the number of animals is too small to draw a firm conclusion on this point
The doses selected for the main teratology study were 100, 500, 1000 g/kg/day. The high dose was chosen to be 1000 mg/kg/day "due to the volume and viscosity of the dose formulation (Vol 1.25, p.131)".

4. AN ORAL TERATOLOGY STUDY OF RENAGEL IN THE RABBIT

Study Nr. GT-01-TX-15 _____. June 1996. Lot nr. 13224N100. GLP statement provided.

Methods -

New Zealand White rabbits (22/dose group), 5 months old, 2.7-3.9 kg, artificially inseminated, were treated orally, via gastric intubation, at same time each day, with **0, 100, 500, 1000 mg/kg/day** on Gest Days 6-18. Controls received only vehicle (water). Animals received 180g Certified _____ per day. On Gest Day 29, animals were killed, and ovaries and uteri were removed and examined.

Results -

Mortality -

1 control found dead G26
1LD found dead G9
2MD found dead G 6,7
7HD found dead G 6,6,6,7,11,12,17
(7 HD's were all replaced)
Clinical signs -
1 LD and 1HD found dead: labored breathing before death)

BW - (data from n = 21to20-21to20-16-22to19) No effects

FC - (data from n = 21to20-21to20-16-22to19) No effects

Gross pathology -

All treatment-related findings were seen in animals that died:
Control: dark discoloration of uterus/vagina, and dark material in uterus/vagina
LD: multiple dark areas in lung, pale material in trachea
MD: dark areas in lung, stomach, sc tissue, thymus; pale material in trachea lumen
HD: dark areas in lung, trachea, esophagus, sc tissue, salivary gland, thymus; pale material or dark fluid in oral cavity and/or trachea; skeletal muscle clot in one animal
(Cause of death is unclear; Dose-dependence of mortality and absence of pathology in HD that survived suggests gavage-related problems)

Reproductive findings:

Number inseminated 22-22-22-29
Number pregnant 22-21-17-23
Pregnancy rate 100-96-81-89% (how calculated?)

Uterine findings (data from n = 20-20-16-19)

Corpora lutea - Not affected (mean # range 10-10.4)
Total number of implantation sites - Not affected (7.4-8.2)
Sex ratio fetuses - No significant effect
Dead fetuses - None 0-0-0-0-0
Live fetuses - No effect (6.3-7.9)
Total number of resorptions - Increase (0.5-0.3-0.5-1.2), not statistically significant, due to increase in early resorptions (see Tables).
Number of empty implantation sites - No effect (0-0-0-0)
Preimplantation loss - No significant effect (17.9-28.8%)
Postimplantation loss - No significant effect (3.1-14.5%).
Uterine weight - No effect
Fetal weights - Increased in female F1 from HD

Table. Effect on resorptions/postimplantation loss

	total implantation sites	early resorptions	middle resorptions	late resorptions	total resorptions	postimplantation loss %	live fetuses
control	7.6	0.4	0	0.2	0.5	6.6	7.1
LD 100 mkd	8.2	0.1	0	0.1	0.3	3.1	7.9
MD 500 mkd	8.1	0.4	0	0.1	0.5	6.1	7.6
HD 1000 mkd	7.4	1.1↑	0.1	0.0	1.2↑	14.5↑	6.3↓

	total nr. of animals	nr. of animals with early resorptions	number of resorptions
control	20	5 (25%)	2-1-2-1-1
LD	20	2 (10%)	1-1
MD	16	4 (25%)	1-3-1-1
HD	19	6 (32%)	1-6-3-4-2-4

Abnormalities: (Fetuses examined 142-159-121-119; Litters examined 20-20-16-19)

No major malformations

No minor anomalies

Conclusions

In the rabbit, Renagel did not cause embryolethality or teratogenicity at doses between 100 and 1000 mg/kg/day. However, a small although not statistically significant increase in postimplantation loss, due to an increased number of early resorptions was observed in the 1000 mg/kg/day dose group.

The highest dose was selected on the basis of the viscosity and volume of the dosing solution. Apparently there were problems with the dose administration by gavage, since there was a dose-related increase in incidence of deaths probably by gavage errors.

GENOTOXICITY

1. SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY

Study nr. GT-01-TX-4. . September 1994. Lot nr. 3V-187-002.

Purpose

Investigate mutagenic potential of extracts of Renastat in an in vitro bacterial mutagenesis assay

Methods

Experimental: Renastat (4g) was presaturated with 40 ml of 0.9% NaCl to form a solid gel. This gel was extracted in 20 ml of NaCl at 50°C for 72h. Bacteria were exposed to test article extract with and without metabolic activation. Test was standard direct plate incorporation assay. Test done at four different dilutions 1:1 (undiluted/"neat"), 1:2, 1:4, 1:8. Plating done in triplicate. Incubation 65h, 37°C. Revertant colonies counted. Test repeated once. No range finding study done.

Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537.

Metabolic activation system: S9 prepared fresh on day of assay (source: liver from Aroclor-treated SD rats)

Test article: Renastat™

Vehicle control: 0.9% NACl

Positive controls:

	-S9	+S9
TA98	2-nitrofluorene (1 ug/plate)	2-aminoanthracene (0.5 ug/plate)
TA100	sodium azide (10 ug/plate)	2-aminoanthracene (1 ug/plate)
TA1535	sodium azide (0.5 ug/plate)	2-aminoanthracene (2 ug/plate)
TA1537	9-aminoacridine (80 ug/plate)	2-aminoanthracene (3 ug/plate)

Doses tested: Renastat extract 1:1 (undiluted/"neat"), 1:2, 1:4, 1:8

Criterion for positive result: A significant increase over negative control values (of >2x) in the mean number of revertants per plate, which was concentration-dependent (all strains). Criterion not entirely clear: apparently is a combination of a significant increase for one concentration, with an otherwise dose-dependent response.

Results

1. Compound tested was extract of Renastat (4g in 20ml final volume). No range finding test was done. Cytotoxicity and solubility not tested.
2. Positive controls all positive (>2x negative control). All results with test article negative: numbers of revertants was not increased as compared to negative controls, for any strain, at any dose.

Conclusion

Under conditions of the assay, Renastat extract is not mutagenic in the Salmonella bacterial mutagenesis test.

2. REVERSE MUTATION TEST OF PB-94 IN BACTERIA

Study nr. TX95-217.
September-December 1995. GLP statement included.

Purpose

Investigate mutagenic potential of extracts of PB-94 in an in vitro bacterial mutagenesis assay.

Note: This test assayed both PB-94 polymer and soluble impurities present in the preparation. Test done by _____ described previously (GT-01-TX-04) only assayed extractable, or soluble, dose components. Sponsor states polymer is hardly absorbed. However, absorption of small amounts eg through phagocytosis is possible.

Methods

Experimental: PB-94 is not soluble in water, DMSO or acetone at levels up to 1mg/ml. Therefore, test article was suspended (disperesed) in DMSO by sonication and mixing. Test was by done by preincubation method. Bacteria were exposed to test article, vehicle or positive control, with and without metabolic activation, for 20 min before plating. Plating was done in duplicate, and in triplicate for negative control. Incubation time >48h, 37°C. Revertant colonies counted. Test repeated once. Sterility test with test substance or S-9 mix only, without bacteria, was also carried out. Preliminary dose-finding assay was done with 313, 625, 1250, 2500, 5000 ug test article/plate.

Doses tested (main test): 313, 625, 1250, 2500, 5000 ug/plate

Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537.

E.Coli: WP2uvrA

Metabolic activation system: S9 fraction of liver homogenate from rats treated with phenobarbital and 5,6-benzoflavone

Test article: PB-94

Vehicle control: DMSO

Positive controls:

	-S9	+S9
TA98	2-nitrofluorene (1ug/plate)	2-aminoanthracene (0.5 ug/plate)
TA100	N-ethyl-nitro-nitrosoguanidine (ENNG) (3 ug/plate)	2-aminoanthracene (1 ug/plate)
TA1535	sodium azide (0.5 ug/plate)	2-aminoanthracene (2 ug/plate)
TA1537	ICR-191 (1 ug/plate)	2-aminoanthracene (2 ug/plate)
E.Coli	ENNG (2 ug/plate)	2-aminoanthracene (10 ug/plate)

Criteria for positive result: A significant increase over negative control values (of >2x) in the mean number of revertants per plate, and dose-dependency (all strains). Criterion not entirely clear: apparently a combination of a significant increase for one concentration, with an otherwise dose-dependent response.

Results

Preliminary test:

1. No effect on # revertant colonies of PB-94 at any dose.
2. No anti-bacterial activity (toxicity) of PB-94 up to 5000 ug/plate.
3. At doses ≥1250 ug/plate insoluble orange-colored PB-94 precipitate visible.
4. Highest dose selected: 5000 ug/plate.

Main test:

1. No effect of test article on #revertants of his or trp genes, with or without S-9.

2. No antibacterial activity at any dose.
3. Precipitates of PB-94 visible at doses ≥ 1250 ug/plate.
4. Positive controls clearly positive (>2x negative control).
5. Sterility test negative.
6. Reproducibility test same results.

Conclusion

Under the conditions of the assay, PB-94 is not mutagenic in the Salmonella and E.Coli bacterial mutagenesis test.

3. IN VITRO MAMMALIAN CYTOGENETIC TEST

Study nr. GT-01-TX-11._____. May-October 1996. Lot nr. 13223NI00. GLP and QA statement provided.

Purpose

Evaluate clastogenic potential of Renagel based upon its ability to induce chromosomal aberrations in Chinese hamster ovary (CHO) cells.

Methods

Experimental: Assay performed using standard procedure. Treatment was carried out in duplicate flasks by adding 0.5 ml dosing /control solution and 4.5 ml medium to seeded cells. In the initial assay cells were exposed to test/control article for 20h (-S9), or for 4h and harvested after 20h (+S9) (1.5x cell cycle). An untreated control was also done. In the independent repeat assay exposure/harvest time were as follows:

-S9		+S9	
exposure (h)	harvest (h)	exposure (h)	harvest (h)
4	20	4	20
20	20	4	44
44	44		

Colcemid was added to cultures 2h before cell collection. Cell growth inhibition was evaluated concurrently for all non-activated and activated assays. Metaphase cells were harvested, fixed in suspension and airdried on glass slides. Stain was 5% Giemsa. Minimal 200 metaphase spreads (100/duplicate flask) per dose level were scored. Cytotoxicity ie cell growth inhibition was measured in initial and repeat assay. Mitotic index was % cells in mitosis per 500 cells counted. Calculated were percent structurally or numerically aberrant cells and mean structural aberrations per cell. Chromatid gaps were recorded but not included in aberration calculations.

Aberrations scored:

Aberration		Types		
Structural	Chromatid-type	breaks	exchanges figures	gaps
	Chromosome-type	breaks	dicentric	ring
Numerical		polyploidy	endoreduplication	

Cells: CHO-K1 cells (ATCC).

Doses tested:

	-S9	+S9
Initial assay	untreated, water (0), 0.5, 1.5, 5, 15, 50, 150, 500, 1500, 5000* ug/ml	untreated, water (0), 0.5, 1.5, 5, 15, 50, 150, 500, 1500, 5000* ug/ml

Repeat assay	untreated, water (0), 79, 157, 313, 625, 1250, 2500, 5000* ug/ml	untreated, water (0), 79, 157, 313, 625, 1250, 2500, 5000* ug/ml

*Highest dose level for which aberrations were scored was level above which test article precipitation on slides or lack of scorable metaphase cells occurred.

Metabolic activation system: S9 fraction of liver homogenate from male SD rats treated with a single ip injection of Aroclor 1254, 500 mg/kg. S-9 prepared and stored at <-70°C until use.

Test article: Renagel (white crystalline powder)

Solvent control: water

Positive controls:

-S9: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (2ug/ml)

+S9: Benzo[α]pyrene2-aminoanthracene (30 ug/ml)

Statistical tests: Fisher's exact test for pairwise comparison treated-control: Cochran-Armitage test for dose-responsiveness.

Criteria for positive result: Increase in % cells with aberrations in dose-responsive manner, with 1 or more concentrations significantly increased relative to solvent control. A reproducible and significant increase at a single dose level over negative control was also considered positive.

Criteria for valid test:

% cells with structural aberrations in negative controls ≤6%.

Positive controls statistically increased over solvent or untreated control

Results

NOTE: The dose levels scored were the highest levels at which there were sufficient scorable metaphase cells, and no excessive test article precipitation on the slides.

Initial assay:

Test article formed workable suspension in treatment medium at concentrations above 150 ug/ml, and was soluble at levels of ≤50ug/ml.

Osmolarity, pH:

Treatment medium 5000 ug/ml: 312 mosmol/kg, pH 8.5

Medium with solvent (water): 295 mosmol/kg, pH ...?

1. -S9, 20h/20h (levels scored 50, 150, 500, 1500 ug/ml): Cell growth inhibition 52%, mitotic index reduction 76% at 1500 ug/ml. No test article-related positive findings.
2. +S9, 4h/20h (levels scored 150, 500, 1500, 5000 ug/ml): Cell growth inhibition 23%, mitotic index reduction 76% at 5000 ug/ml. No test article-related positive findings.

Repeat assay:

Test article formed workable suspension in treatment medium at concentrations above 157 ug/ml, and was soluble at levels of ≤79ug/ml.

Osmolarity, pH:

Treatment medium 5000 ug/ml: 308 mosmol/kg, pH 8.5

Medium with solvent (water): 295 mosmol/kg, pH ...?

1. -S9, 4h/20h (levels scored 625, 1250, 2500): Cell growth inhibition 54%, mitotic index reduction 69% at 2500 ug/ml. No test article-related positive findings.
2. -S9, 20/20h (levels scored 625, 1250, 2500): Cell growth inhibition 55%, mitotic index reduction 63% at 2500 ug/ml. No test article-related positive findings.
3. -S9, 44/44h (levels scored 313, 625, 1250): Cell growth inhibition 57%, mitotic index reduction 68% at 1250 ug/ml. No test article-related positive findings.

4. +S9, 4/20h (levels scored 1250, 2500, 5000): Cell growth inhibition 27%, mitotic index reduction 44% at 5000 ug/ml. No test article-related positive findings.
5. +S9, 4/44h (levels scored 1250, 2500, 5000): Cell growth inhibition 46%, mitotic index reduction 60% at 5000 ug/ml.

Findings:

a) % Cells with structural aberrations significantly increased above concurrent solvent control at 1250, 2500, 5000 ug/ml, all doses, tested.
b) % Cells with structural aberrations significantly increased above untreated control at 1250, 5000 ug/ml.
c) Statistically significant positive dose-responsive trend in % cells with structural aberrations.
d) Type of aberrations increased above control: chromatid breaks > chromosome breaks > dicentric chromosomes > chromatid exchanges. Increase in chromatid breaks and dicentric chromosomes was dose-dependent, other two types were not.
e) % Cells with numerical aberrations not increased.

Table 1. Aberrations per cell (x1000) (mean of duplicate flasks)

	exp/harvest (h/h)	control		treated			positive control
		untreated	water	dose 1	dose 2	dose 3	
-S9	4/20	10	35	20	25	75	240
	20/20	5	0	5	10	20	410
	44/44	25	20	35	55	20	430
+S9	4/20	0	5	5	10	5	760
	4/44	20	5	70[a]	65[a]	85[a]	660

[a] statistical significance not determined

Table 2. % Cells with structural aberrations (mean of duplicate flasks)

	exp/harvest (h/h)	control		treated			positive control
		untreated	water	dose 1	dose 2	dose 3	
-S9	4/20	1	3.5	2	2	5.5	15.5*
	20/20	0.5	0	0.5	1	1.5	27.5*
	44/44	2.5	2	3	3.5	1.5	20.5*
+S9	4/20	0	0.5	0.5	1	0.5	35*
	4/44	1.5	0.5	6*	4.5*	6.5*	32*

*significant increase, and significant dose-response

Table 3. Number of chromatid breaks (mean of duplicate flasks)

	exp/harvest (h/h)	control		treated			positive control
		untreated	water	dose 1	dose 2	dose 3	
-S9	4/20	1	1	0	2	6.5	11.5
	20/20	0	0	0	1	2	16.5
	44/44	1	1	3	4	1.5	16
+S9	4/20	0	0.5	0.5	0.5	0.5	22
	4/44	0	0	3	3	5	22.5

Conclusion

1. Test valid.

2. Test for structural chromosome aberrations was positive for Renagel.
3. The structural aberrations that were induced in a dose-dependent manner were mainly chromatid breaks.
4. The positive result in the presence but not absence of S-9 suggest that some metabolic activation is involved in the generation of the clastogen.
5. The positive result at the 44h but not 20h harvest time could mean that the clastogenic chemical (or another chemical in the test article) induces mitotic delay, or is clastogenic only when cells have passed through more than 1 cell cycle since treatment.
6. The absence of a clear dose-response is in accordance with a mitotic delay induced by the clastogen.
7. Under test conditions Renagel induces structural chromosomal aberrations in cultured mammalian somatic cells.

4. MICRONUCLEUS CYTOGENETIC ASSAY IN THE MOUSE

Study nr. GT-01-TX-12. _____. May-October 1996. Lot nr. 13223NI00. GLP and QA statement provided.

Purpose

Evaluate clastogenic potential of Renagel based upon its ability to induce micronucleated polychromatic erythrocytes in bone marrow of male and female mice.

Methods

Animals/dosing:

ICR mice, 6-8 weeks old, were administered 2 doses of Renagel by IP injection, 24h apart.

Dose levels:

Pilot assay: **1, 10, 1000** (2 males/dose group), **or 5000 mg/kg/day** (5/sex/group)
Toxicity assay: **500, 1000, 2000, 3000, 4000, 4500 mg/kg/day** (5/sex/group)
Micronucleus assay: **0, 572, 1144, 2286 mg/kg/day** (2x5/sex/group). Positive control in main assay was CP, 60/mg/kg/day (5/sex/group). High dose in main assay was based on toxicity assay, and was approximately 0.8x $LD_{50/3}$).

Dose volume: 20 ml/kg.

Vehicle: corn oil.

Observations:

Pilot assay: clinical signs and body weight, daily for 3 days.
Toxicity assay: signs, body weight, daily for 3 days
Main assay: Bone marrow from femur collected at 24h (5/sex/group, and 5/sex/positive control) and 48h (5/sex/group) after last dose administration. Bone marrow cell suspension spread on glass slide (2-4 slides/mouse), fixed with methanol, stained with May-Gruenwald-Giemsa.

Micronuclei scoring:

1000 polychromatic erythrocytes (PCE) scored for presence of micronuclei (round, darkly staining nuclear fragments). Proportion of PCE/total erythrocytes per 1000 erythrocytes also recorded (indicates proliferation state of bone marrow, ie, toxicity marker).

Criteria for positive result:

Treatment-related increase in incidence of micronucleated PCE's, and one or more doses statistically significantly increased over vehicle control at any sampling time. If single dose group increased significantly, test is considered suspect and repeat assay recommended.

Criteria for valid test:

Vehicle control <5/1000 PCE/totalE (0.5%). Positive control significantly increased over vehicle control.

Results

Pilot assay

Mortality: 3/5m and 4/5f at 5000mkd, cause unclear

Signs: Lethargy in 1000mkd males; lethargy and piloerection in 5000 mkd (m+f)

Toxicity assay

dose (mg/kg/day)	mortality		BW change in % (Day3 postdose)	
	m	f	m	f
500	0/5	0/5	1.5	2.5
1000	0/5	0/5	1	-0.5
2000	1/5	1/5	-8	-8
3000	4/5	3/5	-20	-9
4000	4/5	4/5	-12	-9
4500	5/5	4/5	-	-24
5000	3/5	4/5	-11	-9

Various signs (lethargy, piloerection, crusty eyes) more in m than in f, at all dose levels.

Micronucleus assay

Mortality: 1/10 2286mkd males

Signs: Lethargy in m and f, all doses; Crusty eyes in m and f, 2286 mkd.

Micronucleus formation: No significant increase in incidence of micronucleated PCE's in treated groups over vehicle control. Positive control significantly increased.

Treatment dose (mg/kg/day)	Sampling time (h)	n	PCE/1000E	PCE/1000E (change from control,%)	micronucleated PCE/1000PCE
Vehicle	24	5m+5f	0.64	-	1.5
572	24	5m+5f	0.57	-10	0.6
1144	24	5m+5f	0.54	-12	1.0
2286	24	5m+5f	0.55	-14	1.1
CP (60)	24	5m+5f	0.33	-48	44.2
vehicle	48	5m+5f	0.55	-	1.0
572	48	5m+5f	0.57	3	1.0
1144	48	5m+5f	0.54	-2	0.9
2286	48	5m+5f	0.47	-16	1.3

Conclusion

Test was negative:

Under the conditions of the test, Renagel did not induce micronucleated polychromatic erythrocytes in bone marrow of male and female mice.
Extent of exposure of bone marrow to Renagel is unclear. Substance is unlikely to be absorbed into systemic circulation from the peritoneal cavity. On the basis of mortality and clinical signs observed in the three assays (pilot, toxicity, micronucleus) it can not be established whether there is compound-specific toxicity due to systemic exposure. Lack of exposure would render test invalid.