F_1 Generation

1. Maternal and fetal data: As shown in the following table, there were no treatment-related effects on dams and fetuses.

Summary of maternal and fetal data in a Segment III reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 250	UDCA 1000	UDCA 2000
# Dams examined	21	21	22	21
# Implantations/dam	12.48	12.24	12.91	12.62
# Dams that delivered	21	21	22	21
# Live fetuses/ litter	11.71	11.57	12.23	11.71
Mean fetal weight (g)	5.3	5.3	5.1	5.1

2. Postnatal Development: There were no treatment-related effects on eruption of incisor, separation of eyelid, descent of testis and opening of vagina.

3. Organ Weights: There were no treatment-related effects.

4. External Anomalies and Skeletal Variations: Sponsor reported that there were no external anomalies in any fetuses; no data was provided. As shown in the following table, there were no treatment-related skeletal variations at 11 weeks after birth.

Summary of skeletal variations in a Segment II reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 250	UDCA 1000	UDCA 2000
# Fetuses examined	35	32	46	29
Skeletal variations (# of fetuses)				
Fused sternebrae	5	2	4	4
Extra sternebrae	1	0	1	0

5. Reproductive Performance: There were no treatment-related effects on the reproductive performance of the F_1 generation.

In summary, orally administered UDCA did not produce any perinatal and postnatal toxicity in rats.

PROPOSED TEXT OF THE LABELING FOR URSO™ TABLETS:

The sponsor has proposed the following text for the **Carcinogenesis, mutagenesis, impairment of fertility** section of the labeling:

Carcinogenicity, Mutagenicity and Impairment of Fertility

The reviewer is suggesting the following revised version for the **Carcinogenesis, mutagenesis, impairment of fertility** section of the labeling:

The sponsor has proposed the following text for the **Pregnancy Category B** section of the labeling:

The reviewer is suggesting the following revised version for the **Pregnancy Category B** section of the labeling:

The sponsor has proposed the following text for **the Overdose** section of the labeling:

From a preclinical viewpoint, there are no recommended changes for this section of the labeling.

SUMMARY AND EVALUATION:

The pathogenesis of primary biliary cirrhosis (PBC) involves the evolution of (1) inflammation of medium-sized bile ducts, (2) periportal fibrosis, (3) progressive scarring, and (4) firm, regular, intensely bile-stained cirrhosis. Signs and symptoms of PBC usually include pruritus and/or nonspecific fatigue; enlarged, firm, nontender liver; jaundice; cholestasis with elevation of alkaline phosphatase; elevated serum bile acid concentrations and activity of serum γ-glutamyl transpeptidase; possible elevation of serum cholesterol; and antibodies against a component of the inner membrane of mitochondria.

The etiology of PBC is not clear. The presence of antibodies against a component of the inner membrane of mitochondria have prompted many clinicians to view PBC as an autoimmune disease. Thus, historically, attempts have been made to treat PBC with immune modulators such as azathioprine, cyclosporine, chlorambucil and prednisone. These agents were relatively ineffective or too toxic. Currently, liver transplantation appears to the only viable treatment for PBC.

The signs and symptoms of PBC suggest that bile acids might either play a role in the pathogenesis of PBC or might produce symptoms of PBC that could be reduced by modifying physiological and biochemical aspects of bile acids through pharmacological treatment. The sponsor has referred to published clinical studies which suggest that ursodeoxycholic acid (UDCA) has significant therapeutic effects in patients with PBC. Beneficial effects of a pharmacological agent for PBC might include any reduction of the signs and symptoms of PBC such as alleviation of pruritus, enlarged liver and jaundice; the reversal of cholestasis; and the reduction of serum and bile levels of any contributing bile acid and its metabolites. Thus, the sponsor is proposing to market URSO™ (250 mg film-coated tablets of UDCA) for the treatment of PBC.

In support of the NDA application, sponsor has provided preclinical pharmacology studies; absorption, distribution, metabolism and excretion studies in mice, rats and monkeys; acute toxicity studies in mice, rats, hamsters and dogs; a 5-week i.p. toxicity study in rats; 6-month oral toxicity studies in rats; 6-month and 1-year oral toxicity studies in monkeys; 104-week dietary carcinogenic studies in CD-1 and B6C3F$_1$ mice; 104-week and 126-138 week dietary carcinogenic studies in Fischer 344 and Sprague-Dawley rats, respectively; mutagenic studies (Ames test, forward mutation assay in mouse lymphoma cells, sister chromatid exchange assay in human lymphocytes, chromosomal aberrations assay in mouse germ cells, micronucleus test in Chinese hamster bone marrow cells, and chromosomal aberrations assay in Chinese hamster bone marrow cells); 2 Segment I. oral fertility and

reproductive performance studies in rats; 2 Segment II. i.p. teratology studies in mice and rats; 5 Segment II. oral teratology studies in mice, rats and rabbits; and 1 Segment III. oral perinatal and postnatal reproductive toxicity study in rats.

I.v. infusion of ursodeoxycholic acid (UDCA) in rats increased bile flow, bile acid levels, and biliary transport maximum (BSP T_m) of sulfobromophthalein. I.v. administration of UDCA in dogs increased bile flow. I.v. administration of UDCA in rabbits increased bile volume and HCO_3^- concentration. Since cholestasis is a major problem in PBC, any UDCA-induced increase in bile flow, bile acid levels and biliary transport maximum should be beneficial.

When bile ducts of rats were experimentally drained and obstructed, and the mice were intravenously infused (35 μmol/ 100 g) with either taurocholate (TC), taurochenodeoxycholate (TCDC) or tauroursodeoxycholate (TUDC); increases of hepatic and serum alkaline phosphatase were associated with retention of TC and TCDC, but not TUDC. The cholestasis in PBC is usually associated with elevation of alkaline phosphatase. Thus, treatment of PBC with UDCA, which is rapidly absorbed and conjugated in the liver with taurine, should alleviate the elevated alkaline phosphatase indirectly by reversing cholestasis.

After oral administration in mice and rats, UDCA is readily absorbed, primarily by passive diffusion. First-pass metabolism of UDCA in the liver is almost entirely by conjugation with taurine to form tauroursodeoxycholate. During repetitive enterohepatic cycling, some of the UDCA and tauroursodeoxycholate is 6β-hydroxylated to β-muricholate and tauro-β-muricholate in the liver. β-muricholate in the rat is converted by 7β-dehydroxylation to hyodeoxycholic acid, but not in the mouse. In the intestine, tauroursodeoxycholate is deconjugated by bacteria to UDCA, which is 7β-dehydroxylated to form lithocholic acid. Lithocholic acid is not absorbed from the colon in mice and rats. Thus, UDCA and its metabolites are excreted primarily in the feces in mice and rats. In man, most of the administered UDCA is 7β-dehydroxylated to form lithocholic acid and is mainly excreted in the feces; lithocholic acid is partly absorbed and sulfated in the liver. Sulfated lithocholic conjugates (sulfolithocholyl taurine) and sulfolithocholyl glycine) are poorly absorbed from the small intestine and excreted in feces.

In acute oral toxicity studies of UDCA, doses up to 10 g/kg in mice, 5 g/kg in rats and 10 g/kg in dogs were not lethal. The minimum lethal dose of UDCA in hamsters was 1.47 g/kg. Since it has been reported that UDCA administration in hamsters leads to increased bile levels of lithocholic acid, this may explain why hamsters are more sensitive to UDCA than mice or rats. In mice

and rats, orally administered UDCA was immediately followed by slight sedation. In hamsters, orally administered UDCA produced ataxia, inhibition of mobility, dyspnoea, ptosis, decreased food consumption, and body weight loss. In dogs, oral doses of 5.04 g/kg and greater produced salivation and vomiting. Thus, in these cases, dogs most likely did not receive full intended doses.

In a 5-week i.p. toxicity study of UDCA (0, 62.5, 125, 250 and 500 mg/kg/day) in rats, the no effect dose was 62.5 mg/kg/day. Higher doses of UDCA (125 to 500 mg/kg/day) produced deaths, decreases in body weight, increased spleen weights, adhesion of intestines, ascites in abdominal cavity, and increases in incidence of liver histopathological lesions. The liver was a target organ for toxicity.

In a 5-week oral toxicity study of UDCA (0, 0.5, 1.0, 2.0 and 4.0 g/kg/day) in rats, the no effect dose was 4 g/kg/day. Target organs of toxicity were not identified; higher doses of UDCA would need to be studied in order to delineate target organs of toxicity.

In a 6-month oral toxicity study of UDCA (0, 100, 500 and 2500 mg/kg/day) in rats, doses of 500 mg/kg/day and less were well-tolerated. The 2500 mg/kg/day dose produced body weight loss in males and females, and decreased lung weight in females. There were also treatment-related basophilic deposits in kidneys of males and females. Target organs of toxicity may include kidneys; higher doses of UDCA would need to be studied in order to delineate target organs of toxicity.

In another 6-month oral toxicity study of UDCA (0, 0.5, 1, 2 and 4 g/kg/day) in rats, the no effect oral dose was 0.5 g/kg/day. There were treatment-related incidences of intrahepatic cholangitis, hyperplasia of bile ducts and multiple focal liver necrosis in the 1.0, 2.0 and 4.0 g/kg/day groups. Furthermore, the 4.0 g/kg/day dose also produced lethality, reduced body weight and increased organ weights of brain, thyroid glands and adrenal glands. The liver was a target organ of toxicity.

In a 6-month oral toxicity study of UDCA (40 and 100 mg/kg/day) in monkeys, UDCA was well-tolerated. UDCA inhibited HMG-CoA reductase activity and produced proliferation of the smooth endoplasmic reticulum in the liver.

In a 1-year oral toxicity study of UDCA (0, 50, 100, 300 and 900 mg/kg/day) in monkeys, the no effect dose was 50 mg/kg/day. Higher doses (100, 300 and 900 mg/kg/day) produced mortality, body weight loss, decreased food consumption, increased serum LAP and bilirubin levels, and increased liver weights. The 300 and 900 mg/kg/day doses also produced gross pathological lesions (cloudy swelling of liver) and histopathological lesions

(increased numbers of lysosomes and cell necrosis in liver; proliferation of epithelial cells, inflammation and necrotic cells in bile ducts); thus, the liver was a target organ for toxicity. Finally, UDCA was more toxic in the monkey after 1-year treatment than after 6-months treatment.

In a 104-week dietary carcinogenic study [redacted] Report # 406-006) of UDCA (0, 25, 150 and 1000 mg/kg/day) in CD-1 mice, the incidence of kidney adenocarcinomas in males of the high-dose group (3/50) was within the background range of incidence (0-6.0%) of kidney adenomas and adenocarcinomas in Charles River CD-1 male mice. No kidney adenocarcinomas were seen in females of any dosage group. The range of background incidence of kidney adenomas in Charles River CD-1 female mice is 0-1.4%. There were no other treatment-related incidences of neoplastic lesions. The sponsor did not provide any explanation for the dosage selection. However, the recommended maximum feasible dose (5%) was exceeded in males (7.19%) and females (6.44%) in the 1000 mg/kg/day dosage group.

In a 104-week dietary carcinogenic study [redacted] Report # 536) of UDCA (0, 300, 900 and 2,700 ppm) in $B6C3F_1$ mice, there were no treatment-related incidences of neoplastic lesions. Mean achieved doses for males over 104 weeks were 37.4, 116 and 362 mg/kg/day for the 300, 900 and 2,700 ppm doses, respectively. Mean achieved doses for females over 104 weeks were 49.4, 146 and 459 mg/kg/day for the 300, 900 and 2,700 ppm doses, respectively. The sponsor stated that dosage selection was based upon preliminary results that indicated appropriate growth rate suppression; details were not provided. However, there were no treatment-related effects on body weight in the present study. On the other hand, there were time-related and treatment-related increased incidences of bile duct dilation and hyperplasia. These doses (116 and 362 mg/kg/day in males; 459 mg/kg/day) in females) represent maximally tolerated doses.

In a 103-week oral carcinogenic study of lithocholic acid (125 and 250 mg/kg/day 3 times a week) in $B6C3F_1$ mice, there were no treatment-related incidences of neoplastic lesions.

In a 104-week dietary carcinogenic study [redacted] Report # 537) of UDCA (0, 500, 1,700, and 5,000 ppm) in Fischer 344 rats, there were no treatment-related incidences of neoplastic lesions. Mean achieved doses for males over 104 weeks were 22.5, 77.2 and 239 mg/kg/day for the 500, 1,700 and 5,000 ppm doses, respectively. Mean achieved doses for females over 104 weeks were 28.5, 97.5 and 300 mg/kg/day for the 500, 1,700 and 5,000 ppm doses, respectively. The sponsor stated that dosage selection was based upon preliminary results that indicated appropriate growth rate suppression; details were not provided. In the present study, mean body weights of males and females in the 5,000 ppm group were reduced by 8-10% during Weeks 65-104,

respectively. Furthermore, the highest dose of UDCA in the diet was 5%. According to guidelines published in the Federal Register in 1995 [60 FR 11278], the maximum feasible dose by dietary administration is considered to be 5% of the diet.

In a 126-138-week dietary carcinogenic study (LPT) UDCA (0, 33, 100 and 300 mg/kg/day) in Sprague-Dawley rats, there was a treatment-related increase in benign adrenal pheochromocytomas in females; 8/50 females in the 300 mg/kg/day dosage group had adrenal pheochromocytomas, compared to 2/50 females in the control group. Dietary administration of UDCA was continued until a mortality rate of approximately 70% was reached in the control groups. There were no other treatment-related incidences of neoplastic lesions. The sponsor stated that dosages of UDCA were selected with the purpose of reaching subtoxic levels for at least 2 of the 3 dosages; no further information was provided. Sponsor did not achieve the recommended maximum feasible dose (5%) by dietary administration. Furthermore, there is no evidence that a maximally tolerated dose was achieved. Any utilization of plasma AUC ratios for dosage selection of UDCA does not seem reasonable because of the extensive enterohepatic cycling of UDCA and its taurine and glycine conjugates.

In a 103-week oral carcinogenic study of lithocholic acid (250 and 500 mg/kg/day 3 times a week) in Fischer 344 rats, there were no treatment-related incidences of neoplastic lesions.

Although intrarectally administered lithocholic acid and taurodeoxycholate (1 mg/kg 5 times weekly for 13 months) did not produce any tumors in the distal colon and rectum of Fischer 344 rats, both compounds promoted N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced colonrectal neoplasms. MNNG alone produced neoplasms in 25% of the animals. MNNG + lithocholic acid produced neoplasms in 52% of the animals, while MNNG + taurodeoxycholate produced neoplasms in 62% of the animals. Thus, lithocholic acid alone and taurodeoxycholate alone were not carcinogenic, but both compounds promoted MNNG-induced colonrectal neoplasms.

In another study, cholic acid (0.2% and 0.4% in the diet) dose-dependently promoted the incidence of azoxymethane (AOM)-induced colonic tumors in Fischer 344 rats. On the other hand, 0.4% UDCA decreased the incidence of AOM-induced colonic tumors. 0.4% UDCA also dramatically increased the ratio of benign/malignant tumors; that is, completely abolished malignant tumors.

UDCA was not mutagenic in the Ames test, forward mutation assay in mouse lymphoma cells, sister chromatid exchange assay in human lymphocytes, chromosomal aberrations assay in mouse germ cells, micronucleus test in Chinese hamster bone marrow cells, and chromosomal aberrations assay in Chinese hamster bone marrow cells. In the Ames test, concentrations of DMSO were not optimal

during metabolic activation experiments. Thus, UDCA was not mutagenic without metabolic activation, but no conclusions can be made about the experiments with metabolic activation.

In a Segment I oral fertility and reproductive performance study of UDCA (0, 300, 900 and 2,700 mg/kg/day; vehicle was 0.8% aqueous hydroxypropylmethylcellulose solution) in Sprague-Dawley rats that was performed at [redacted], UDCA did not affect fertility and general reproductive performance of the F_0 generation.

In a Segment I oral fertility and reproductive performance study of UDCA (0, 250, 1000 and 2,000 mg/kg/day; vehicle was 0.5% gum arabic solution) in Wistar rats that was performed at [redacted] UDCA significantly reduced the number of pregnant females in the 2000 mg/kg/day dosage group. Moreover, UDCA produced a decrease in the number of live fetuses/litter at the 2000 mg/kg/day dose and a dose-related retardation of fetal skeletal ossification. Since UDCA produced dose-related decreases in body weights of dams, the effects of UDCA on number of pregnant females in the 2000 mg/kg/day dosage group and the toxic effects of UDCA on fetuses are probably related to general UDCA-induced toxicity.

In a Segment II i.p. teratogenic study of UDCA (0, 30 and 200 mg/kg on Day 7 through Day 12 of gestation; vehicle was 1% carboxymethyl cellulose solution) in dd mice that was performed at [redacted] UDCA was not teratogenic. Since organogenesis in mice occurs from approximately Day 6 through Day 15 of pregnancy, dosing in this study did not completely cover the period of organogenesis. Furthermore, summaries, but no detailed data, were provided for fetal anomalies and variations.

In a Segment II oral teratogenic study of UDCA (0, 300 and 1,500 mg/kg/day on Day 7 through Day 12 of gestation; vehicle was 1% carboxymethyl cellulose solution) in dd mice that was performed at [redacted] UDCA was not teratogenic. Since organogenesis in mice occurs from approximately Day 6 through Day 15 of pregnancy, dosing in this study did not completely cover the period of organogenesis. Furthermore, summaries, but no detailed data, were provided for fetal anomalies and variations.

In a Segment II i.p. teratogenic study of UDCA (0, 30 and 200 mg/kg on Day 9 through Day 14 of gestation; vehicle was 1% carboxymethyl cellulose solution) in Wistar rats that was performed at [redacted] UDCA was not teratogenic. Since organogenesis in rats occurs from approximately Day 6 through Day 17 of pregnancy, dosing in this study did not completely cover the period of organogenesis. Furthermore, summaries, but no detailed data, were provided for fetal anomalies and variations.

In a Segment II oral teratogenic study of UDCA (0, 300 and 4,000 mg/kg/day on Day 9 through Day 14 of gestation; vehicle was 1% carboxymethyl cellulose solution) in Wistar rats that was performed at [redacted] UDCA was not teratogenic. Since organogenesis in rats occurs from approximately Day 6 through Day 17 of pregnancy, dosing in this study did not completely cover the period of organogenesis. Furthermore, summaries, but no detailed data, were provided for fetal anomalies and variations.

In a Segment II oral teratogenic study of UDCA (0, 250, 1000 and 2000 mg/kg/day on Day 7 through Day 17 of gestation; vehicle was 0.5% gum arabic solution) in Wistar rats that was performed at [redacted] UDCA was not teratogenic.

In a Segment II oral teratogenic study of UDCA (0, 33, 100 and 300 mg/kg/day on Day 6 through Day 18 of gestation; vehicle was 0.8% aqueous hydroxypropylmethylcellulose solution that was performed at [redacted] in White Russian rabbits, UDCA was not teratogenic. However, UDCA did produce maternal toxicity. The 100 and 300 mg/kg/day doses reduced maternal body weight and food consumption; the 300 mg/kg/day dose was lethal. However, detailed data were not provided for gross pathology and for fetal anomalies and variations.

In a Segment II oral teratogenic study of UDCA (0, 5, 10 and 20 mg/kg/day on Day 6 through Day 18 of gestation; vehicle was 0.5% gum arabic solution) in New Zealand white rabbits performed at [redacted] UDCA was not teratogenic. Finally, detailed data were not provided for gross pathology and for fetal anomalies and variations.

In a Segment III oral perinatal and postnatal reproductive toxicity study of UDCA (0, 250, 1000 and 2000 mg/kg/day on Day 17 of gestation through Day 21 of lactation; vehicle was 0.5% gum arabic solution) in Wistar rats that was performed at [redacted] UDCA did not produce any perinatal and postnatal toxicity.

Thus, sponsor has provided preclinical pharmacological and pharmacokinetic studies of orally administered UDCA which support its intended use for the treatment of PBC. Preclinical toxicity studies indicated that the liver is the primary target organ of toxicity for orally administered UDCA in several species; including mice, rats, dogs and monkeys; in all cases, liver toxicity occurred at oral UDCA doses that were many-fold higher than proposed therapeutic doses. Carcinogenic studies of UDCA were negative in CD-1 mice, $B6C3F_1$ mice, and Fischer 344 rats. There was a treatment-related increase in benign adrenal pheochromocytomas in female Sprague-Dawley rats. The UDCA metabolite lithocholic acid was not carcinogenic in $B6C3F_1$ mice

and Fisher 344 rats, but did promote N-methyl-N'-nitro-N-nitrosoguanidine-induced colonrectal neoplasms in Fischer 344 rats. *In vitro* and *in vivo* mutagenic studies of UDCA were all negative. Orally administered UDCA did not affect fertility and general reproductive performance in Sprague-Dawley rats and Wistar rats; was not teratogenic in dd mice, Wistar rats, White Russian rabbits, and New Zealand white rabbits; and did not produce any perinatal and postnatal toxicity in Wistar rats. Therefore, from a preclinical viewpoint, orally administered UDCA appears to be approvable for the treatment of PBC.

Finally, the reviewer has suggested a revised version for the **Carcinogenesis, mutagenesis, impairment of fertility** section and the **Pregnancy** section.

RECOMMENDATIONS:

From a preclinical viewpoint, the NDA is approvable.

/S/ [redacted] 1/9/97

Gerald A. Young, Ph.D.
Pharmacologist

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-180/Dr. Young
HFD-345/Dr. Viswanathan
HFD-101/CAC/Dr. DeGeorge WOC II, Room 6014

R/D Init.: J. Choudary 10/29/96

GAY/hw/11/19/96 & 12/24/96
C:\WPFILES\PHARM\N\20675611.0GY

(1) Noted.

(2) See the accompanying Pharmacology Team Leader's Addendum. (Pages 150a to 150g)

/S/ [redacted] 1/17/97

Attachments:	Page Numbers
Appendix I:	151 - 164
Appendix II:	165 - 208
Appendix III:	209 - 247
Appendix IV:	248 - 265

NDA 20,675

URSO™ (ursodiol)
Tablets, 250 mg

Pharmacology Team Leader's Addendum
To Dr. G.A. Young's Pharmacology Review
of January 9, 1997

1. Noted.

2. The quoted doses for cholic acid (1.2 and 2.4 g/kg/day) and ursodiol (1.2 and 2.4 g/kg/day) on page 101 of the review are inaccurate. The correct doses for both compounds are 120 and 240 mg/kg/day.

3. Carcinogenicity Potential:

Oral carcinogenicity studies of ursodiol were conducted in CD-1 mice (US), $B6C3F_1$ mice (Japan), Fischer 344 rats (Japan) and Sprague-Dawley rats (Germany). The drug was administered via diet. With few exceptions, histopathological examination of tissues were confined to high dose group and control group in each study. Dose selections in these studies were appropriate and the studies were adequate.

a. In the two-year carcinogenicity study in CD-1 mice, doses of 25, 150 and 1000 mg/kg/day were employed. No tumorigenic potential was manifested in this study. The observed incidence of renal adenocarcinoma in 3 or 50 male mice of the high dose group should be considered in the context of the high background incidence of nephrosis (72 to 92%) and the incidence of renal adenocarcinoma in 1 of 50 males of concurrent control group. The differences in the incidences between the groups were apparently not statistically significant.

b. In the two-year carcinogenicity study in $B6C3F_1$ mice, doses of 37-49, 116-146 and 362-459 mg/kg/day were tested. There was no evidence for tumorigenic potential in this study.

c. In the two-year carcinogenicity study in Fischer 344 rats, doses of 23-29, 77-98 and 239-300 mg/kg/day were employed. There was no evidence for tumorigenic potential in this study.

d. The Sprague-Dawley rat carcinogenicity study was a life-span study, i.e. longer than two years. Animals were treated with doses of 33, 100 and 300 mg/kg/day until the mortality in the control animals reached 70%. Accordingly, the male segment of the study was terminated after 126 weeks and the female segment was terminated after week 138. Histopathology examination of all tissues was limited to all high dose animals and only 50% of the control animals. Kidneys, adrenals and urinary bladders of the low and mid dose group animals were also examined. There was a

significant ($p \leq 0.05$, Fisher's Exact test by sponsor) increase in the incidence of adrenal medullary pheochromocytoma in the females of 300 mg/kg/day group (8 of 50 animals) when compared to the control group females (1 of 50 animals). The incidence was, however, within the strain historical background incidence range of 2.4 to 18.5%. As expected, there was a general increase in the incidence of progressive renal disease. There was a treatment related increased incidence of "calcareous albuminous and crystalline deposits in pelvis and pelvic mucosa" in both sexes. Increased incidences of "mineralized concretions" were also found in female Sprague-Dawley rats of an earlier two-year carcinogenicity study of ursodiol under a different NDA. During weeks 127 to 138, there were occurrences of "hypernephroid" carcinoma of the kidney in 1 female rat of 33 mg/kg/day group and 3 female rats of 300 mg/kg/day group but none in the control or 100 mg/kg/day group. There were no such tumors in the male rats. Because the histopathology examination of the tissues in the concurrent control animals was limited to 50% of the animals only, the strain historical background incidence of this tumor needs to be taken into consideration. The historical background incidence for renal cell carcinoma in 102-week old female Sprague-Dawley strain rats was 2 to 3.6%. Since the observed incidence of the renal tumors in the treated animals was only in 127-138 weeks of the study but not earlier, it may be interpreted as the consequence of the superimposition of reported test compound precipitation in the kidneys on the top of the observed age related general increase in the progressive renal disease at two years of age. The incidence of "hypernephroid" carcinoma of the kidney in females rats of 300 mg/kg/day group was not statistically significant by sponsor's statistical analysis (Fisher's Exact test).

e. The results of the above four carcinogenicity studies did not demonstrate a carcinogenicity risk to humans from the chronic use of ursodiol. The observed statistically significant increase in the incidence of adrenal medullary pheochromocytoma in the 300 mg/kg/day group female Sprague-Dawley rats is not relevant to human risk because of its poor predictive value (Diener and McClain, Symposium: Adrenal Gland Toxicity and Neoplasia, J. Am. Coll. Tox., 7:1, pp 1-109, 1988). The results of the carcinogenicity studies of ursodiol in mice and rats under this NDA are also in general in concordance with the results of previous carcinogenicity studies of ursodiol under a different NDA. The increased incidence of adrenal medullary pheochromocytoma in rats should be incorporated in the labeling for this NDA.

f. The results of National Cancer Institute sponsored oral (gavage) carcinogenicity studies of lithocholic acid, (a metabolite of ursodiol) in B6C3F$_1$ mice (125 and 250 mg/kg/day, 3 times/week for 103 weeks) and Fischer 344 rats (250 and 500 mg/kg/day, 3 time/week, for 103 weeks) did not provide any evidence for carcinogenic potential. Intrarectal administration of lithocholic acid (1 mg/kg/day, 5 times/ week, 13 months) to Fischer 344 rats in a 78-week study (Narisawa et al, J. Natl. Cancer Inst. 53:1093-1095, 1974) was also not carcinogenic. However, its administration following rectal treatment with a single dose of a known carcinogenic agent (N-methyl-N'-nitro-N-Nitrosoguanidine, 4 mg/kg) enhanced the incidence of colorectal tumors as evidenced by 25% incidence in the animals receiving carcinogen alone and 52% in animals receiving both treatments. This tumor promoting effect of lithocholic acid should, however, be weighted with the known inhibitory effect of dietary ursodiol (240 mg/kg/day for 28 weeks) on the carcinogenic effect of two weekly subcutaneous injections of azoxymethane (15 mg/kg) in male Fischer 344 rats (Earnest et al, Cancer Research 54:5071-5074, 1994). The incidence of colonic tumors was 47% in azoxymethane treated rats while the incidence was 22% in the rats receiving the combined treatment of azoxymethane and ursodiol. The average ratio of benign to malignant tumors was also dramatically reduced. A Balanced summary of the above findings should also be included in the labeling.

4. Genotoxicity: Ursodiol was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y, TK$^{+/-}$) forward mutation test, the human lymphocyte sister chromatid exchange test, the mouse spermatogonia chromosome aberration test, the Chinese hamster micronucleus test and the Chinese hamster bone marrow cell chromosome aberration test.

5. Reproductive Toxicity: Teratologic potential of ursodiol was assessed in one intraperitoneal and one oral study in mice, one intraperitoneal and two oral studies in rats and two oral studies in rabbits. In none of the above studies it manifested any teratogenic potential. In the two studies in mice and two of three studies in rats, the drug was not administered during the entire period of organogenesis. These studies should be excluded from the labeling.

6. RECOMMENDATIONS:

a. Pharmacology recommends approval of this application.

b. The marked portions in the attached sponsor's version of labeling should be replaced by the following:

I. PRECAUTIONS

a. "Carcinogenesis, Mutagenesis, Impairment of Fertility:

b. "Pregnancy. Teratogenic Effects. Pregnancy Category B:

[redacted]

/S/ [redacted] 2/4/97

Jasti B. Choudary, Ph.D., B.V.Sc.
Pharmacology Team Leader

Attachment: Marked copy of sponsor's draft labeling.

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-180/Dr. Young
HFD-345/Dr. Viswanathan

JBC/hw/2/3/97
N:\WPFILES\PHARM\N\20675701.0JC

12 Pages
Draft
Labeling

Appendix I

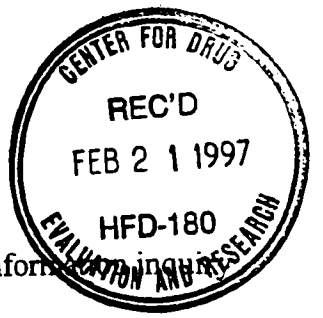
Incidence summaries for non-neoplastic and neoplastic histopathology lesions in a 2-year carcinogenicity study in CD-1 mice

[redacted] Report # 406-006)

100 pages REDACTED
RAW DATA

FEB 19 1997

STATISTICAL REVIEW AND EVALUATION
CARCINOGENICITY



NDA#	NDA 20-675
Applicant	Axcan Pharma U.S, Inc.
Name of Drug	URSO™ (Ursodiol) Tablets
Indication:	Treatment of Primary Biliary Cirrhosis
Document Reviewed	Applicant's letter of response to the FDA information inquiry of 4/23/1996 NDA Tumorigenicity Study Data/Hard copy and Diskette dated 7/9/1996
Statistical Review	
Primary	Yi Tsong, PhD
Secondary	Mohammad Huque, PhD, Nancy Smith, PhD
Pharmacologist	This review has been discussed with the primary pharmacological reviewer, Gerald Young, PhD

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1. Introduction

The purpose of this report is to evaluate the study of carcinogenic potential on Ursodiol, submitted by Axcan Pharma, Inc. The analyses, as a response to the FDA requested, were based on data of two mouse and two rat studies provided by sponsor. To assess the dose-response relationships, the animal survival and tumor data were analyzed. The analyses were done by species, by study and by sex.

The carcinogenicity analyses consist of two parts, the survival data analyses and the tumor data analyses. The purpose of the survival data analyses were: (1) to examine the significance of the differences in survival among the treatment groups (i.e. homogeneity test); (2) to determine the significance of positive or negative dose-mortality trend (does-mortality trend test.) In the tumor data analysis, the tumors were classified as either fatal (lethal) or non-fatal (non-lethal) type. In the analysis for a selected tumor, the significance of dose-tumor positive linear trend was of primary interest. The reviewer applied the death-rate method to fatal tumors and prevalence method to non-fatal tumors. Both of them were referred as exact test in the following context. For tumors that caused death for some, but not for all rats, a combined test was performed. The combined test used the Z-statistic which was assumed to follow a standard normal distribution. This test was referred to as the asymptotic test in the following context.

The reviewer's decision on significance of trend for tumors that were either fatal or non-fatal to all rats relied on p-values of exact test. For other tumors, the p-values of asymptotic test were used. The p-values in the parentheses were not used in determination but for reference. The Office of Epidemiology and Biometrics, CDER/FDA uses a rule similar to the Haseman rule for multiple comparisons. This rule says that in order to keep the false positive rates at an overall nominal level of approximately 0.1, tumor types with a spontaneous tumor rate of $\leq 1\%$ (rare tumor) as shown in data should be tested at 0.025 significance level, otherwise (common tumor) a 0.005 significance level should be used.

A pairwise comparison between any one of dose treatment groups and placebo treatment group will be used to identify whether the difference is significant between each of the dose treatment groups and placebo group. CDER/FDA uses the rule for significant ruling by testing at 0.01 significance level for common tumor and at 0.05 significant level for rare tumor.

2. The Rat Studies

2.1 Chronic Toxicity and Carcinogenicity Study with Ursodeoxycholic Acid in Rats (Study 200)

2.1.1 Study Design

To assess the carcinogenic potential of ursodiol, the sponsor used 719 rats (360 males and 359 females) of Fischer 344 strain produced by [redacted]. For each sex, the rats were divided randomly into four treatment groups with equal number of rats, with dose levels of 0 (control), 500, 1700 and 5000 ppm per day during the entire 104 weeks feeding period. The rats were treated by dietary administration. In the reviewer's analyses, the 40 interim sacrificed rats in each dose group were excluded as requested by pharmacologist with 200 males and 199 females used for the carcinogenicity assessment. The number of rats by dose and sex are given in the following table.

		Dose Level (ppm)				Total
		0 (Control)	500	1700	5000	
Sex	Male	50	50	50	50	200
	Female	49	50	50	50	199
Total		99	100	100	100	399

All cages were inspected once daily for any mortality, and all the animals were physically examined at least once per week palpable masses. All survival rats were necropsied and microscopically examined at the end of week 104.

The survival data and tumor data were provided by sponsor. The data were stored on one 3 1/2 floppy diskette.

The Reviewer's Analyses

Details of the reviewer's analyses are described in the Appendix A in this report. Tables and Figures are identified by their page number such as A-M-1, A-M-2, etc for males and A-F-1, A-F-2, etc for females.

2.1.2 Survival Data Analysis

Tables A.M.1 and A.F.1 describe the number of male rats and female rats which died during the experimental period, by dose and time, in intervals, respectively and the interval mortality rates for males and females, respectively. Tables A.M.2 and A.F.2 describe the survival rates of males and females, respectively. Figures A.M.3 and A.F.3 are the cumulative percentages of death of males and females, respectively of the four treatment groups. In males, the death rates of the four treatment groups were very close

with slightly higher rates in high dose group in the last interval. In females, the high dose group had the highest rates in the last two intervals, while the medium dose group had the lowest rates. Kaplan-Meier survival curves of males and females are also given in Figures A.M.3 and A.F.3 respectively. In males, the four treatment groups had no apparent difference. In females, the difference appeared to be between week 50 and week 80. Both high and low dose groups appeared to have lower survival rates than others. However, the difference was not consistent and disappeared after week 80.

The following table describes the p-values from the homogeneity test of survival and from the dose-mortality trend test. For either sex, the differences among the treatment groups were not significant. Also, the dose-mortality trends were not significant either.

Test for homogeneity			
Sex	Male	Method	p-value
		Cox	0.5372
		Kruskal-Wallis	0.5218
	Female	Cox	0.5014
		Kruskal-Wallis	0.4786
Test for Dose-Mortality Trend			
Sex	Male	Method	p-value
		Cox	0.2424
		Kruskal-Wallis	0.2395
	Female	Cox	0.1428
		Kruskal-Wallis	0.1554

2.1.3 Tumor Data Analyses

The reviewer performed the dose-tumor positive linear trend tests using the exact permutation test for all the fatal and non-fatal tumors. When the tumor was fatal to some but all rats, a combined asymptotic test was used.

Tables A.M.5 and A.F.5 describe all the organ-tumors categories tested. The p-values of the exact and asymptotic procedures for the trend test are given in Tables A.M.6 and A.F.6. The reviewer's decision on significance of trend for tumors that were either fatal or non-fatal to all rats relied on p-values of exact test. For other tumors, the p-values of asymptotic test were used. The p-values in the parentheses were not used in determination but for reference. The Office of Epidemiology and Biometrics, CDER/FDA uses a rule similar to the Haseman rule for multiple comparisons. This rule says that in order to keep

the false positive rates at an overall nominal level of approximately 0.1, tumor types with a spontaneous tumor rate of ≤1% (rare tumor) as shown in data should be tested at 0.025 significance level, otherwise (common tumor) a 0.005 significance level should be used.

In this study, pathological examination was not performed on all organs in the low and medium dose groups. Since trend test results were meaningful only for the tumors that had patholologically examined, the results of pairwise comparisons between the high dose and control groups were used in these cases. In both males and females, none of the p-values shown were statistically significant.

2.1.4 Reviewer's Comments

In this study, there was no evidence to indicate the carcinogenicity effect of treatment of ursodiol among the male and female rats.

2.2 Carcinogenicity Study with Ursodeoxycholic Acid, Batch no. 110 7987-Called for Short "UDCA" - in Sprague-Dawley Rats at Administration in the Food [redacted] Study).

2.2.1 Study Design

To assess the carcinogenic potential of ursodiol, the sponsor collected the data of 400 rats (200 males and 200 females) of Sprague-Dawley strain. The study was carried out by the [redacted] For each sex, the rats were divided randomly into four treatment groups of equal number of rats, with dose levels of 0 (control), 33, 100 and 3000 mg/kg/day during the study. Seventy percent of the males died before the final kill at the 126th week. In females, 70% died before the final kill at the 138th week. The rats were treated by dietary administration. The number of rats by dose and sex are given in the following table.

		Dose Level (mg)				Total
		0 (Control)	33	100	300	
Sex	Male	50	50	50	50	200
	Female	50	50	50	50	200
Total		100	100	100	100	400

All cages were inspected once daily for any mortality, and all the animals were physically examined at least once per week palpable masses. All survival rats were necropsied and

microscopically examined at the end of study.

The survival data and tumor data were provided by sponsor. The data were stored on one 3 1/2 floppy diskette.

The Reviewer's Analyses

Details of the reviewer's analyses were described in the Appendix B in this report. Tables and Figures are identified by their page number such as B-M-1, B-M-2, etc for males and B-F-1, B-F-2, etc for females.

2.2.2 Survival Data Analysis

Tables B.M.1 and B.F.1 describe the number of male rats and female rats which died during the experimental period, by dose and time, in intervals respectively and the interval mortality rates for the males and females respectively. Tables B.M.2 and B.F.2 describe the survival rates of males and females respectively. Figures B.M.3 and A.F.3 are the cumulative percentages of death of males and females, respectively of the four treatment groups. In males, the death rates of the control and high dose treatment groups were greater than the low and medium dose groups after 78 weeks of treatment. In females, no difference was shown among the four treatment groups. Kaplan-Meier survival curves of males and females are also given in Figures B.M.3 and B.F.3 respectively. In males, the low and medium dose groups had higher survival rates than the other two groups between approximately week 60 and week 120. The difference was consistent till the end of the study. In females, the ursodiol treated groups had lower survival rates than the control group after 120 weeks of treatment.

The following table describes the p-values from the homogeneity test of survival and from the dose-mortality trend test. For either sex, the differences among the groups were not significant. Also, the dose-mortality trends were not significant either.

Test for homogeneity			
Sex	Male	Method	p-value
		Cox	0.5014
		Kruskal-Wallis	0.4786
	Female	Cox	0.3015
		Kruskal-Wallis	0.6677

Test for Dose-Mortality Trend			
Sex	Male	Method	p-value
		Cox	0.1428
		Kruskal-Wallis	0.1554
	Female	Cox	0.4827
		Kruskal-Wallis	0.5169

2.2.3 Tumor Data Analyses

The reviewer performed the dose-tumor positive linear trend tests using the exact permutation test for all the fatal and non-fatal tumors. When the tumor was fatal to some but all rats, a combined asymptotic test was used. Tables B.M.5 and B.F.5 describe all the organ-tumors categories tested. The p-values of the exact and asymptotic tests are given in Tables B.M.6 and B.F.6 for trend test. The reviewer's decision on significance of trend for tumors that were either fatal or non-fatal to all rats relied on p-values of exact test. For other tumors, the p-values of asymptotic test were used. The p-values in the parentheses were not used in determination but for reference. The Office of Epidemiology and Biometrics, CDER/FDA uses a rule similar to the Haseman rule for multiple comparison. This rule says that in order to keep the false positive rates at an overall nominal level of approximately 0.1, tumor types with a spontaneous tumor rate of $\leq 1\%$ (rare tumor) as shown in data should be tested at 0.025 significance level, otherwise (common tumor) a 0.005 significance level should be used.

In the trend test for males, there was no tumor showing a significant positive trend.

In the trend test for females, there were two tumor types showing a significant positive linear trend. The trend test for adrenal pheochromocyte results in a significant p-value of 0.0017 (as compared with the cut-off value of 0.005 for common tumor). The pairwise comparison between the high dose and control group resulted in a p-value of 0.0046, which was also significant (as compared with cut-off value of 0.01 for common tumor). The trend test for kidney hypernephroid resulted in a significant p-value of 0.0107 (as compared with the cut-off value of 0.025). The pairwise comparison between the high dose and control groups resulted in a significant p-value of 0.0248 (as compared with the cut-off value of 0.05).

Sex	Tumor Type	Tumor Incidence				p-value	
		Cntl	Low	Medium	High	Exact	Asymp
F	Adrenal Pheochromocyte	1/50	3	2	8	(0.0032)	0.0017
		1/50			8/50	(0.0101)	0.0046
	Kidney Hypernephroid	0/50	1	0	3	(0.0263)	0.0107
		0/50			3/50	(0.0834)	0.0248

2.2.4 Reviewer's Comments

There were evidences of carcinogenicity effect of treatment of ursodiol among the female rats in this study. In female rats, significant positive dose-tumor trend were shown in adrenal pheochromocyte and kidney hypernephroid.

In contrast to the length (104 weeks) of a standard rat study, this study is an extended study which started the final kills at 138th week instead of 104 weeks because it took 138 week in the study to observe 70% death. Therefore many tumors were developed after 104 weeks. For example, the three rats in the high dose group had kidney hypernephroid at 127, 131 and 138 week, respectively.

In addition to the issue on extended length of the study, the validity of the study is yet to be addressed for the reason that the sponsor reported and provided data of 50 male and 50 female rats in the control group instead of the 100 rats in each sex when the study started. It was not documented what was the selection criterion of the 50 rats reported and whether bias was introduced because of the selection criterion.

3. The Mouse Studies

3.1 Carcinogenicity Study with Ursodeoxycholic Acid (UDCA) in Mice (Study No. 199)

3.1.1 Study Design

To assess the carcinogenic potential of ursodiol, the sponsor used 640 mice (320 males and 320 females) of $B6C3F_1$ strain. The study was carried out by [redacted] as Study 199. For each sex, the mice were divided randomly into four treatment groups of equal number of mice, with dose levels of 0 (control), 300, 900 and 2700 ppm per day during the entire 104 weeks feeding period. The mice were treated by dietary administration. The reviewer's analyses were carried out with 200 male and 200 female mice, excluding