Toxicity Studies Summary:

Segment I Studies

1) **Time-Course and Reversibility of Ribavirin-Induced Testicular Degeneration in the Mouse**, Study #:066, GLP

Study Initiation: 7 Mar. 1990, Ribavirin Lot #04200787 (D17),
2) **Ribavirin: A Pilot Study Of Toxicity To Male Rats By Oral Administration For 9 Weeks**, Study # INP 6/931480, GLP,
Study Initiation: 27 Jan. 1993, Ribavirin Lot #05500787 (R-17),
3) **Ribavirin: Effect On Fertility And General Reproductive Performance Of The Rat**, Study # INP 8/932344, GLP,
Study Initiation: 17 June 1993, Ribavirin Lot #05500787 (R-17),

Segment II Studies

4) **The Effect of Virazole on Pregnancy of Rat**, Study ID INP 4/911237, GLP, Study Initiation: 13 Sept., 1991,
Ribavirin Batch # 05500787 (R-17).
5) **The Effect of Virazole on Pregnancy of Rabbit**, Study ID INP 5/911238, GLP, Study Initiation: 13 Sept., 1991,
Batch # 05500787 (R-17).

Segment III Studies

6) **Ribavirin: The Effect On The Pregnant Rat And Offspring During The Peri- and Post-Natal Period**, Study # INP 7/931920, GLP,
Study Initiation: 26 March 1993, Ribavirin Lot# 05500787 (R-17),

Toxicity Study Reviews:

Segment I Studies

1) **Time-Course and Reversibility of Ribavirin-Induced Testicular Degeneration in the Mouse**, Study #:066 (Task Order #:UIC-7,
Status: GLP
Testing Site(s):

Study Initiation: 7 March 1990
Compound Tested: Ribavirin, Lot# 04200787 (D17), purity unspecified.
Doses Tested: 0, 35, 75 or 150 mg/kg/day
Dose Volume and Route: 15 ml/kg, gavage
Solvent: distilled water
Species, Strain, Sex: male CD-1 mice age 6 weeks, mean weight 29.8 grams, 50 or 70 animals /dose.
Test Procedure: A total of 240 male CD-1 mice were randomly assigned to treatment groups using a stratified procedure based on body weight. Seventy animals each were assigned to the control and high dose groups, while 50 animals each were randomized to the 2 other dose groups. Animals were dosed daily. Animals in the high dose group received drug daily for 3 months, while animals in the low and medium dose groups received drug on a daily basis for 6 months. Drug solutions were prepared every 2-3 weeks, and were assayed for

ribavirin concentration by an All drug solutions were reported within 10% of the nominal concentration.

Observations made periodically before and throughout the study included: body weight (non-fasted) and food consumption (once per week and at scheduled termination), physical examination (once per week), morbidity-mortality and general activity-appearance (twice daily, before dosing in the morning and again in the mid-afternoon). Blood samples were obtained from 10 animals/group at each of the following time-points: 1, 2, 3, 4.5, and 6 months following initiation of drug administration. Blood samples were obtained from the orbital sinus, and were analyzed

Mortality: Surviving animals were sacrificed and necropsied according to the schedule presented in the following table. All animals which died on study (2 control animals and 3 animals each in the 75 and 150 mg/kg dose groups) or were sacrificed moribund (3 low dose and 1 high dose animal) were necropsied. Most deaths were apparently related to gavage trauma, with no apparent ribavirin induced mortality among the test animals. Euthanasia was performed by carbon dioxide inhalation, following which the testes, prostate, epididymides, pituitary, brain, seminal vesicles, and adrenal glands were collected. The left testis and epididymides were separately weighed, fixed (Bouins solution and 10% buffered formalin, respectively), stained with hematoxylin and eosin, and examined microscopically (including analysis of seminiferous tubule diameter). The right testes and epididymides were collected for sperm evaluation (sperm and spermatid counts, sperm motility, and sperm morphology). At scheduled sacrifice, blood samples were obtained for determination of RBC micronuclei.

Summary Schedule of Terminal Sacrifices

Dose mg/kg	# of animals	Sacrifice Date (in Months) 1	2	3	4.5	6	9	12
0	70	10	10	10	10	10	10	10
35	50	-	-	10	10	10	10	10
75	50	-	-	10	10	10	10	10
150[14]	70	10	10	10	10	10	10	10

Body Weight and Clinical Signs: A loss of body weight (0.5 g loss from baseline) was observed among the animals in the 150 mg/kg dose group during the first week of drug administration (mean body weight at end of treatment week 1: control = 32.2 grams, ribavirin treated = 30.7 grams), but was followed by increased body weight gains during the next 2-3 weeks (comparable body weight for control [34.3 g] and 150 mg/kg dose [34.2 g] groups at 28 days of drug administration). Animals in the 35 and 75 mg/kg dose conditions were significantly smaller (failure to gain weight) than the controls on a nearly continuous basis between weeks 6-11 and 14-16 of drug treatment. Throughout the remainder of the dosing interval, body weights among these animals were reduced relative to that of the controls (non-significant differences). Food consumption was not reported. Poor grooming and rough coat hair were the only other clinical signs observed among the ribavirin treated animals and occurred at all doses, but primarily in the 150 mg/kg dose group (15 of 70 animals).

Gross Pathology: There were no significant differences between the absolute

[14] Due to excessive toxicity among animals from the high dose group, ribavirin treatment at this dose was discontinued after 3 months. Ribavirin administration at the low and intermediate doses was continued for the scheduled 6 months.

weight of the testes or epididymides for any of the treatment groups. However, there was a tendency to reduced weight of the testes among treated animals in the 150 mg/kg dose group (after 3 months of treatment), which was statistically signifi-cant when testicular weight was expressed as a percent of the body weight (0.66 and 0.57% of body weight for the control and high dose groups, respectively). Frequency of RBC micronuclei were not significantly affected by ribavirin treatment at any dose.

Microscopic Pathology: Microscopic examination of the testes revealed an increased incidence of germinal epithelial vacuoli-zation (control = 1/10, 35 mg/kg = 2/10, 75 mg/kg = 5/10, and 150 mg/kg = 5/10 animals, after 3 months of treatment; 1/10 and 6/10 animals in the 150 mg/kg condition showed similar lesions at 1 and 2 months of treatment, respectively), characterized by swollen cells with clear cytoplasmic vacuoles and marginated nuclei. Reduced thickness of the germinal epithelium was evident in 1/10 animals each in the 35 and 75 mg/kg dose groups (months 4.5 and 6 of treatment, respectively), and 5/10 animals in the 150 mg/kg group (after 3 months of treatment). Necrosis of the testicular germinal epithelium was evident in 1/10 animals in the 75 mg/kg condition at month 3, and 2, 1 and 1/10 animals in the 150 mg/kg condition at 3, 4.5 and 6 months. Epididymal epithelial necrosis was evident in 3/10 animals in the 150 mg/kg dose group at the month 2 sacrifice only.

Reductions in testicular germinal epithelial thickness, along with vacuolization and necrosis, appeared to be potentially reversible as their incidence was reduced among animals from the 150 mg/kg treatment group at 3 months following drug cessation (vacuolization 1/10 [2/10 at 1.5 months post-treatment], reduced epithelial thickness 1/10, and epithelial necrosis 0/10 [1/10 at 1.5 months post-treatment]).

Dose dependent reductions in testicular spermatid counts (up to approximately 40%), and increases in the epididymal content of abnormal sperm (approximately 40% amorphous sperm) were related to the administration of ribavirin (see following table). Sperm concentration in the epididymides and the percent of motile sperm were marginally effected by ribavirin administration only at 150 mg/kg dose. The velocity of sperm movement was reduced, while the linear/lateral movement ratio was increased. Seminiferous tubule diameter was significantly reduced (approximately 8.4%) among animals in the 150 mg/kg treatment group.

Serum FSH, LH and Ts were not significantly altered ($p > 0.05$) by ribavirin treatment. However, mean serum LH and FSH levels among ribavirin treated animals were consistently lower than those of the control animals during months 1 and 2 of testing. (The lack of statistically significant effects was probably due to the small number of samples and the episodic variability of the measured levels. In addition, any drug induced effects were superimposed on a consistent pattern of decreasing levels of FSH, LH and Ts among the control animals during the 6 months of the study.)

Comments: 1) Ribavirin administration appears to produce a progressive and dose dependent toxicity on testicular function in the male CD-1 mouse. Significant decreases in spermatid counts (approx. 40%) and increases in abnormally shaped sperm (approx. 40%) were observed following the administration of ribavirin in all doses tested. The maximal toxic responses were apparently similar for all doses, with dose effects being related to the rate of progression to maximal response.

2) The study failed to identify a No Effect Level for ribavirin induced testicular toxicity.

Summary of Sperm Evaluations

Month	Dose (mg/kg/day) 0	35	75	150
	Sperm Morphology, Mean (S.D.;N), % Abnormal Cells			
1	3 (1;9)	--	--	30 (15;10)
2	3 (2;10)	--	--	44 (16;10)
3	2 (1;10)	18 (12;9)	29 (14;10)	38 (18;10)
4.5	3 (3;10)	25 (18;10)	41 (17;10)	3 (2;10)
6	4 (4;10)	9 (4;10)	11 (5;10)	5 (4;10)
9	3 (2;10)	2 (1;10)	3 (1;10)	4 (3;10)
12	3 (2;7)	2 (1;7)	4 (3;6)	4 (4;6)
	Sperm Concentration, Mean (S.D.;N), 10^6 cells/ml			
1	13 (5;10)	--	--	13 (5;10)
2	16 (5;10)	--	--	15 (6;10)
3	15 (4;10)	17 (4;10)	17 (4;10)	17 (5;10)
4.5	13 (5;10)	17 (4;10)	19 (6;10)	17 (4;10)
6	13 (2;10)	12 (5;10)	12 (5;10)	11 (3;10)
9	15 (3;10)	14 (4;10)	15 (4;10)	17 (3;10)
12	12 (3;7)	12 (5;7)	13 (6;6)	15 (3;6)
	Sperm Motility, Mean (S.D.;N), % Motile			
1	42 (19;10)	--	--	37 (10;10)
2	37 (14;10)	--	--	26 (6;10)
3	45 (15;10)	38 (16;10)	35 (15;10)	34 (11;10)
4.5	56 (12;10)	47 (15;10)	42 (10;10)	55 (11;10)
6	39 (15;10)	44 (12;10)	42 (11;10)	50 (20;10)
9	48 (14;10)	44 (15;10)	48 (8;10)	49 (11;10)
12	33 (17;7)	41 (16;7)	30 (18;6)	40 (14;6)
	Spermatid Counts, Mean (S.D.;N), #/g of testis x 10^8			
1	1.0 (.2;10)	--	--	0.6 (.1;10)
2	1.2 (.1;10)	--	--	0.7 (.2;10)
3	1.4 (.2;9)	1.3 (.2;10)	1.2 (.2;10)	1.1 (.2;10)
4.5	1.3 (.3;10)	1.1 (.2;10)	0.9 (.2;10)	1.3 (.2;10)
6	1.5 (.2;10)	1.0 (.1;10)	0.9 (.2;10)	1.5 (.3;10)
9	1.4 (.2;10)	1.3 (.2;10)	1.4 (.2;10)	1.3 (.2;10)
12	1.7 (.2;7)	1.4 (.3;7)	1.0 (.3;6)	1.5 (.3;6)
	Seminiferous Tubule Diameter, Mean (S.D.;N), mm			
1	.26 (.02;10)	--	--	.27 (.02;10)
2	.28 (.01;10)	--	--	.25 (.02;10)
3	.27 (.01;10)	.28 (.02;10)	.26 (.01;10)	.26 (.02;10)
4.5	.26 (.02;10)	.26 (.01;10)	.26 (.01;10)	.27 (.01;10)
6	.29 (.03;10)	.27 (.02;10)	.27 (.02;10)	.28 (.02;10)
9	.28 (.02;10)	.27 (.02;10)	.29 (.02;10)	.27 (.02;10)
12	.26 (.04;8)	.27 (.02;7)	.26 (.03;6)	.27 (.03;6)

___ = Significantly different from the control group mean

Comments: (continued)

3) No assessments of plasma drug levels, drug induced changes in hematologic parameters, or changes in gross organ pathology (splenomegaly) were included in this study for comparison with the results of previously reported studies. Inclusion of such measures would provide significant cross-study validation of the spectrum, extent and reproducability of the toxicities observed.

4) The results suggest that cessation of drug administration may be associated with a nearly complete recovery of sperm production and normal testicular histology.

5) Failure to demonstrate significant changes in serum levels of FSH, LH and Ts suggests that the observed toxic changes in the testes and sperm may be due to direct effects of ribavirin at these sites.

2) **Ribavirin: A Pilot Study Of Toxicity To Male Rats By Oral Administration For 9 Weeks**, Study # INP 6/931480.
Status: GLP
Testing Site:
Study Initiation: 27 Jan. 1993
Compound Tested: Ribavirin, Lot# 05500787 (R-17), purity 100%
Doses Tested: 0, 40, 80, 160, 320 and 640 mg/kg/day
Dose Volume and Route: 1 ml/100 g, gavage
Solvent: distilled water
Species, Strain, Sex: male Sprague-Dawley rats Crl:CD (SD) BR VAF/Plus), age approx. 6 weeks, weight range 135-163 grams, 5 animals/dose.
Test Procedure: Animals were randomly assigned to treatment groups using a stratified procedure based on body weight. Drug was administered once daily by gavage. Drug solutions were prepared each week, and were assayed for ribavirin concentration All drug solutions were reported within 10% of the nominal concentration.

Observations made periodically before and throughout the study included: body weight (non-fasted) and food consumption (once per week), physical examination (once per week), morbidity-mortality and general activity-appearance (twice daily, pre/post-dosing). Water consumption was measured between days 6 and 33 of dosing. Necropsies were performed on all animals at time of sacrifice, with histologic evaluations of the reproductive organs. Sperm samples were analyzed for concentration and motility.

Mortality, Body Weight and Clinical Signs: All animals dosed at 640 or 320 mg/kg/day were sacrificed in extremis during the initial 2 weeks of treatment. Body weight and food consumption among the affected animals were significantly depressed, although water consumption was increased. Concurrent signs of lethargy, ataxia, piloerection and reduced motor tonus were evident. At necropsy, the affected animals showed decreased adipose tissue, accentuated lobular markings of the liver, reduced size of the seminal vesicles, prostate and testes, and abnormalities of the intestinal contents (green-yellow fluid accumulation, 8/10 animals). One animal dosed at 160 mg/kg/day was found dead during the 3rd week of dosing. There were no other premature deaths during the study, and no abnormal clinical signs evident among the animals dosed at 40-160 mg/kg/day.

Body weight gain and food consumption was reduced among animals treated at 80 and 160 mg /kg/day during the initial 5 weeks of dosing, but was comparable to the controls during the remainder of the study. The changes in body weight and food consumption were concordant, and no change in the efficiency of food utilization was evident. Body weight gain and food consumption was not effected by the administration of ribavirin at 40 mg/kg/day. Water consumption among the animals dosed at 160 mg/kg/day was consistently greater (40%) than that of the controls.

Gross and Microscopic Pathology: Among animals surviving to the termination of the study, slight reductions in the weight of the testes, epididymides, seminal vesicles and prostate were evident, along with enlargement of the heart (2/5) in animals dosed at 160 mg/kg/day. There were no other gross abnormalities which appeared related to the test compound.

Spermatid counts were reduced slightly among the ribavirin treated animals (particularly in the vas deferens) although the effects were not significant due to one control animal with abnormally low values. Sperm motility was comparable among all study groups. There were no microscopic abnormalities noted in the reproductive organs of the ribavirin treated males.

Comments: 1) The study results suggest that oral doses of ribavirin ≥ 320 mg/kg/day, when administered as a single daily dose to male SD rats, exceed the maximum tolerated dose for 9 weeks of administration.

2) Based on these results, doses of ribavirin of up to 160 mg/kg/day were selected for use in the definitive Segment I reproductive toxicity study conducted in male rats.

3) As noted above, abnormalities of adipose (reduced content) and cardiac tissues (cardiomyopathy) were evident in several animals dosed at > 160 mg/kg/day. These abnormalities may be indicative of a metabolic alterations and damage to tissues with a high metabolic demand. All future submissions should be closely monitored for evidence of similar, or related, effects.

3) **Ribavirin: Effect On Fertility And General Reproductive Performance Of The Rat,** Study # INP 8/932344.

Status: GLP
Testing Site:
Study Initiation: 17 June 1993
Compound Tested: Ribavirin, Lot# 05500787 (R-17), purity 100%
Doses Tested: Males: 0, 10, 40 and 160 mg/kg/day
Females: 0, 0.3, 1.0 and 10.0 mg/kg/day
Dose Volume and Route: 1 ml/100 g, gavage
Solvent: distilled water
Species, Strain, Sex: male and female Sprague-Dawley rats Crl:CD (SD) BR VAF/Plus), weight range: males: 185-233 grams; females: 185-226 grams, 15 males and 30 females /dose, additional groups of 5 females/dose were used for the assessment of drug absorption.

Test Procedure: Animals were randomly assigned to treatment groups using a stratified procedure based on body weight. Drug was administered once daily by gavage. Drug administration was continued for 9 weeks prior to mating in the males [dosing was continued among the treated males until all study animals were terminated], and was performed from 2 weeks prior to mating until day 5 of pregnancy (presumed) in the females. Approximately one-half of the drug treated females were allowed to give birth and rear the young to weaning.

Observations made throughout the study included: body weight and food consumption, physical examination, morbidity-mortality and general activity-appearance (pre/post-dosing). Necropsies were performed on all animals at the time of sacrifice (at the conclusion of the study for the males, and on day 13 of gestation or at the time of weaning among treated females).

Dose Preparations: Drug solutions were prepared each week, and were assayed for ribavirin concentration . All drug solutions were within 5% of the nominal concentration.

Mortality and Clinical Signs: A total of 7 animals (6 male; 1 female) died or were sacrificed in extremis during the study. Four male animals from the high dose group died prematurely (2 animals each during weeks 2 and 3 of dosing), while an additional 2 animals were sacrificed during weeks 8 and 16. One female animal (0.03 mg/kg/day) was sacrificed in extremis during the second week of treatment. Signs noted prior to death included; pale extremities, hunched posture, piloerection, decreased body weight and/or weight loss, and

respiratory distress (female). Post-mortem examinations revealed fluid accumulation in the thoracic or abdominal cavities of most of the moribund animals, and fluid in the lungs (suggestive of a gavage accident) of the affected female animal.

An additional 4 male animals treated with ribavirin at 160 mg/kg/day, showed pale extremities on multiple occasions during the dosing interval. All surviving males from the high dose group showed signs of alopecia and scabbing during the final weeks of treatment, with 7/9 animals showing swollen muzzles. There were no abnormal clinical signs noted among males treated at 10 or 40 mg/kg/day, or among females treated at any dose level.

Body Weight and Food Consumption: Body weight gain was reduced during the initial 4 weeks of drug treatment among males from the high dose group, but was unaffected among males dosed at either of the lower doses. Weight gain among the high dose treated males recovered slightly after the initial weeks of dosing, although it remained somewhat depressed throughout treatment. Body weight gain among males treated at 10 or 40 mg/kg/day, and among females from all treatment groups (through day 5 of gestation), was comparable to the concurrent controls. Weight gain among female animals treated at 10 mg/kg/day was slightly reduced after day 5 of pregnancy.

Similar to the changes in body weight, food consumption and the efficiency of food utilization was reduced among males from the high dose group during the first 4 weeks of treatment. Following this period, food consumption and food utilization was generally comparable for all study groups.

Mating Performance and Duration of Pregnancy: There were no differences in the median pre-coital interval or the pregnancy rate of animals treated with ribavirin at any dose level. The duration of pregnancy was comparable for all study groups.

Gross Pathology: There were no treatment related lesions noted among the dams at the time of necropsy.

Plasma Drug Levels: Plasma samples were obtained from groups of 5 satellite animals for each test dose at 1 hour after drug administration on day 5 of pregnancy. The mean plasma drug levels for the control through high dose group were as follows: 0, 0.082, 0.26 and 1.63 μM/ml.

Litter Parameters: There was a slight increase in the incidence of pre-implantation fetal loss among dams dosed at 1 and 10 mg/kg /day. However, there were no differences evident in the litter size, litter weight, mean pup weight, or ratio of male to female offspring at day 13 of gestation or at the time of littering. The viability and growth rate of offspring were comparable for all study groups.

Among the offspring of dams dosed at 10 mg/kg/day and allowed to litter, there were increases in the incidence of hydrocephaly (7 pups from 3/17 litters) and diaphragmatic herniation (3 pups from 1 litter). At 1 mg/kg/day, a total of 2/18 litters contained pups (1 pup in each litter) with hydrocephaly. There were no other adverse effects noted in the offspring of ribavirin treated dams.

Comments: 1) Ribavirin administration at a dose of 160 mg/kg/day resulted in excessive toxicity and death in 6 male animals. However, among the surviving male animals at all treatment levels there were no significant changes in reproductive behavior or fertility.

2) Female reproductive behavior and fertility was not effected by treatment with ribavirin at doses up to 10 mg/kg/day.

3) A slightly increased incidence of hydrocephaly and diaphragmatic herniation was noted among the offspring of females dosed with ribavirin at 10 mg/kg/day.

4) Based on the study results, the estimated NOEL doses for toxicity in the F_0 generation are 10 and 40 mg/kg /day for female and male animals, respectively. For the F_1 generation, the estimated NOEL dose is 1 mg/kg/day.

5) Mean plasma drug levels generally increased in an approximately linear manner with increases in the administered dose of ribavirin.

Segment II Studies

4) **The Effect of Virazole on Pregnancy of Rat**, Study ID INP 4/911237.
Status: GLP
Study Initiation: 13 Sept., 1991
Study Site:

Compound Tested: ribavirin, Batch # 05500787 (R-17)
Doses Tested: 0, 0.3, 1.0, and 10.0 mg/kg/day
Dose Volume and Route: 1 ml/100 g, gavage
Solvent and Control: distilled H_2O
Species, Strain, Sex: Sexually mature female and male Sprague Dawley rats (CRL: CD(SD) Br VAF/plus rats), Female: age 8-10 weeks, weight 177-251 grams at time of randomization; Male: age 8-10 weeks, weight not specified. There were 25 female animals assigned to the low and intermediate dose groups, while 40 animals each were assigned to the control and high dose groups. Breeding was performed by the animal supplier Kent).

Test Conditions: Animals were randomly allocated into 4 groups (25 or 40 females/group). Test compound was administered by gastric infusion once each day between days 6 and 15 of gestation (post-coitus). An additional 5 pregnant female animals/group were administered drug on a similar basis and blood samples were drawn 1 and 24 hours following ribavirin administration on days 6 and 15 of testing for the determination of plasma/erythrocyte drug levels. Mortality, physical signs, body weight and food/water consumption were measured. On day 20 (post-coitus), the dams were sacrificed and given a detailed macroscopic external and internal examination. All fetuses were weighed, sexed, and examined externally for abnormalities. Alternate foetuses were retained for evaluation of soft-tissue anomalies, with the remaining foetuses being used for evaluation of skeletal anomalies.

There were no premature deaths among the female animals in any of the treatment groups. Food and water consumption, and clinical signs were not affected by the administration of ribavirin at any of the doses tested. There were no apparent differences in the incidence of gross pathologies among the treated and control animals of the parent generation at the time of necropsy on day 20 of pregnancy.

Plasma and erythrocyte levels of ribavirin as measured following drug administration of the first (Day 6) and last (Day 15) day of drug administration are presented in Table 1. As is evident, higher levels of ribavirin were detected both in erythrocytes and plasma 1 hour following dosing as compared with 24 hours after drug administration. Further, the levels of ribavirin in plasma and erythrocytes were greater after repeated dosing than after a single dose, with concentrations of drug in erythrocytes being greater than those measured in plasma. All dosing solutions were determined to be within 7% of nominal drug concentrations.

Plasma and Erythrocyte Levels of Ribavirin

Dose mg/kg/day	0.0	0.3	1.0	10.0
Day 6: Plasma Conc.				
1 Hr. Post-Dose	***	0.06	0.10	0.98
24 Hr. Post-Dose	***	***	***	0.08
Erythrocyte Conc.				
1 Hr. Post-Dose	***	0.63	1.66	12.15
24 Hr. Post-Dose	***	0.41	0.73	3.88
Day 15: Plasma Conc.				
1 Hr. Post-Dose	***	0.06	0.11	1.26
24 Hr. Post-Dose	***	***	0.06	0.11
Erythrocyte Conc.				
1 Hr. Post-Dose	***	1.51	2.76	14.92
24 Hr. Post-Dose	***	0.44	0.93	4.33

*** Below level of detection, 0.05 µMoles/litre

Among female animals in the high dose treatment group, body weight gain was reduced beginning at day 12 of pregnancy and continuing to day 20 (mean reduction in weight gain among high dose animals of 14 grams, equal to approximately 10% of the weight gain seen in the control animals; $p<0.01$). However, the reduction in body weight among the high dose treated animals was apparently a reflection of a decrease in fetal survival and mean litter weights, as the adjusted body weights on day 20 (terminal body weight minus litter and uterine weights) were comparable for all treatment groups.

Pregnancy rates and pre-implantation events (i.e., number of corpora lutea and implantation sites) were not affected by the administration of ribavirin (see Tables 2 & 3). There was one incident of a complete litter resorption in a female animal from the high dose group. In contrast, post-implantation fetal loss was significantly increased among females in the high dose group (71 deaths/360 implantations) as compared with the controls (37 deaths/371 implantations), with the majority of the losses (39) in the high dose group occurring late in gestation. Mean viable litter size, litter weight (29.1 grams versus 44.9 grams), and foetal weight (2.8 grams versus 3.8 grams) were reduced among animals in the high dose treatment group as compared with the controls. The ratio of male to female foetuses was comparable among all groups.

Summary of Litter Parameters

Dose mg/kg/day	0.0	0.3	1.0	10.0
No. of Litters	28	20	20	28
Corpora Lutea	14.8	14.7	14.7	14.8
Implants	13.3	12.7	13.3	12.9
Pre-implant Loss %	9.0	12.8	9.9	11.8
Embryonic Deaths				
Early	1.3	0.5	0.5	1.1
Late	0.0	0.0	0.0	1.4**
Total	1.3	0.5	0.5	2.5*
Post-Implant Loss %	10.7	3.4	3.5	20.2*
Live Young (Total)	334	244	255	289
Live Young/Litter	11.9	12.2	12.8	10.3
Mean Litter Wt.	44.9	46.5	48.3	29.1**
Mean Foetal Wt.	3.77	3.86	3.81	2.81**

* $p<0.05$, ** $p<0.01$, Where not specified - values are Mean/Litter

Summary of Pregnancy Rate (# of animals)

Dose mg/kg/day	0.0	0.3	1.0	10.0
Mated (group size)	40	25	25	40
Non-pregnant	12	5	5	11
Total Resorption	0	0	0	1
Dams with Live Foetuses at Termination	28	20	20	28

Incidence of Selected Visceral Anomalies

Dose mg/kg/day	0.0	0.3	1.0	10.0
Foetuses:				
No. Examined	168	123	127	145
Hydrocephaly	---	---	1	10
Retinal Folds	---	---	1	3
Diaphragm Hernia	---	---	---	24
Displaced Adrenal	---	---	---	19
Displaced Oesophagus	--	---	---	8
Vascular Defects				
Atrial Septum	---	---	---	1
Ventricular Sept.	4	1	1	45
Inf. Vena Cava	---	---	---	24
Rt. Azygous Vein	---	---	---	32
Malrotated Heart	---	---	---	4
Litters:				
No. Examined	28	20	20	28
Hydrocephaly	---	---	1	6
Retinal Folds	---	---	1	3
Diaphragm Hernia	---	---	---	16
Displaced Adrenal	---	---	---	13
Displaced Oesophagus	--	---	---	6
Vascular Defects				
Atrial Septum	---	---	---	1
Ventricular Sept.	4	1	1	23
Inf. Vena Cava	---	---	---	16
Rt. Azygous Vein	---	---	---	16
Malrotated Heart	---	---	---	3

Incidence of Selected Skeletal Anomalies

Dose mg/kg/day	0.0	0.3	1.0	10.0
Foetuses:				
No. Examined	166	121	128	144
Abnormal Vertebrae				
Cervical-				
Fusions	---	---	---	6
Thoracic-				
Fusions	---	---	---	9
Irreg. Ossif.	2	1	1	96
Lumbar-				
Fusions	---	---	---	7
Irreg. Ossif.	---	---	---	39
Fused Ribs	---	---	---	22
Scoliosis	---	---	---	7
Extra Vertebra	2	1	11	123

Incidence of Selected Skeletal Anomalies (continued)

Dose mg/kg/day	0.0	0.3	1.0	10.0
Sternum				
Irregularities	---	---	---	20
Irreg. Costal Cart.	---	---	---	49
Split Sternum	---	---	---	6
Limb Defects including Ectrodactyly	---	---	---	4
Malrotated hind Limbs	---	---	---	5
Litters:				
No. Examined	28	20	20	28
Abnormal Vertebrae				
Cervical-				
Fusions	---	---	---	6
Thoracic-				
Fusions	---	---	---	6
Irreg. Ossif.	2	1	1	28
Lumbar-				
Fusions	---	---	---	4
Irreg. Ossif.	---	---	---	19
Fused Ribs	---	---	---	12
Scoliosis	---	---	---	5
Extra Vertebra	1	1	6	26
Sternum				
Irregularities	---	---	---	11
Irreg. Costal Cart.	---	---	---	21
Split Sternum	---	---	---	4
Limb Defects including Ectrodactyly	---	---	---	3
Malrotated hind Limbs	---	---	---	4

In light of the frequency and variety of visceral and skeletal abnormalities observed in the exposed offspring, incidence data for selected abnormalities are presented above, and are listed by the numbers of foetuses and litters effected. Among foetuses from the high dose treated dams, greater that 60% and 90% of the foetuses allocated to visceral and skeletal examinations showed significant abnormalities. Major abnormalities included numerous vascular defects (septum and inferior vena cava), midline closure failures (herniation, displaced oesophagus, split or irregular sternum), and vertebral abnormalities (fused or extra cervico-thoracic-lumbar vertebra and ribs).

Among foetuses from dams treated at 1.0 mg/kg/day, the incidence of skeletal and visceral abnormalities remained elevated versus the concurrent controls, although the incidence was considerable reduced versus that of the high dose group (see tables above). Abnormalities among foetuses exposed to ribavirin at 0.3 mg/kg /day were not detectably different from the control.

Comments: 1) The results suggest that ribavirin at doses between 0.3 and 10.0 mg/kg/day had minimal effects (reduced weight gain) on the course of pregnancy in the female rat. Reductions in weight gain seen among the high dose treated females during and following drug dosing was apparently due to reductions in fetal survival and mean litter weight, as terminal mean body weights [body weight minus the weight of the litter and uterus] were similar for the treatment and control groups.

2) Plasma and erythrocyte levels of ribavirin measured 1 hour following drug administration, demonstrated significant accumulation of drug with repeated dosing, and generally increased

in a dose related manner. At 24 hours following dosing, plasma levels of drug were near or below the limit of detection (0.05 µM/L) in all dose groups, while erythrocyte concentrations remained elevated (demonstrating the accumulation and retention of ribavirin following repeated administration). At 1 hour following oral administration of ribavirin in the pregnant rat (0.3 and 1.0 mg/kg), mean plasma levels of drug ranged from undetectable levels to approximately 0.10 µM [0.024 µg/ml], and decreased to undetectable levels at 24 hours.

3) While maternal toxicities were minimal, significant adverse effects on the developing embryo/foetus were evident among animals from the high and intermediate dose groups. Ribavirin administered at 10 mg/kg/day, significantly (2x) increased the incidence of late foetal deaths as compared to control. At both 1.0 and 10.0 mg/kg/day, ribavirin administration resulted in significant dose related increases in the incidence of gross visceral and skeletal abnormalities (see Tables 4 & 5).

4) No teratogenic effects were evident in the rat administered daily oral doses of 0.3 mg/kg (estimated human equivalent doses of 0.04 mg/kg, based on body surface area adjustment). This dose is considered to define the "No Observable Effects Level" (NOEL) for ribavirin in the rat. Mean plasma levels of drug were near or below the limit of detection (0.05 µM), 1 hour following the administration of 0.3 mg/kg of ribavirin to the pregnant rat.

5) **The Effect of Virazole on Pregnancy of Rabbit**, Study ID INP 5/911238.
Status: GLP
Study Initiation: 13 Sept., 1991.
Study Site:
Compound Tested: ribavirin, Batch # 05500787 (R-17)
Doses Tested: 0.0, 0.1, 0.3 and 1.0 mg/kg/day
Dose Volume and Route: 5 ml/kg, gavage
Solvent and Control: distilled H_2O
Species, Strain, Sex: female New Zealand White rabbits, age 15-24 weeks, weight 2.9-4.0 kg, 16 females/group.
Test Conditions: Animals were randomly allocated into 4 groups (16 females/group). Females were paired one-to-one with males during breeding. Test compound was administered by gastric intubation once each day between days 6 and 18 of gestation (post-coitus). Blood samples were obtained from 6 animals/group on days 6 and 18 of pregnancy (1 and 24 hours after dosing), for the monitoring of plasma and erythrocyte drug levels. Mortality, physical signs, body weight and food/water consumption were measured. On day 29 (post-coitus), the dams were sacrificed and given a detailed macroscopic external and internal examination. All fetuses were weighed, sexed, and examined externally and internally for abnormalities. All foetuses were processed for skeletal anomalies.

Plasma and erythrocyte levels of ribavirin as measured following drug administration of the first (Day 6) and last (Day 18) day of drug administration are presented below. As is evident, higher levels of ribavirin were detected both in erythrocytes and plasma 1 hour following dosing as compared with 24 hours after drug administration. Further, the levels of ribavirin in plasma and erythrocytes were greater after repeated dosing than after a single dose, with concentrations of drug in erythrocytes being greater than those measured in plasma. All dosing solutions were determined to be within 9% of nominal drug concentrations.

Plasma and Erythrocyte Levels of Ribavirin

Dose mg/kg/day	0.0	0.1	0.3	1.0
Day 6: Plasma Conc.				
1 Hr. Post-Dose	***	0.07	0.20	0.80
24 Hr. Post-Dose	***	***	0.06	0.12
Erythrocyte Conc.				
1 Hr. Post-Dose	***	0.87	2.43	7.67
24 Hr. Post-Dose	***	0.36	1.02	2.83
Day 18: Plasma Conc.				
1 Hr. Post-Dose	***	0.11	0.31	1.11
24 Hr. Post-Dose	***	0.05	0.09	0.31
Erythrocyte Conc.				
1 Hr. Post-Dose	***	1.49	4.12	11.83
24 Hr. Post-Dose	***	0.90	2.24	5.52

*** Below level of detection,

There were no premature deaths or overt clinical signs among the treated dams during the course of the study. Food intake was not affected by the administration of ribavirin at any of the doses tested. However, administration of ribavirin at 1.0 mg/kg/day, induced a transient reduction (approximately 35 g) in weight gain (between days 6 and 8), followed by the maintenance of intergroup differences during the remainder of the treatment and gestational period. The difference in weight gain of the control and high dose treated animals averaged 50 grams and sporadically achieved statistical significance ($p<0.05$). Body weight gain of animals in the low and intermediate dose groups did not differ from that of the control animals. There were no apparent differences in the incidence of gross pathologies among the treated and control animals of the parent generation at the time of necropsy.

For the control, low, intermediate and high dose groups, there were a total of 15, 15, 13, and 16 females bearing live young at the time of necropsy on day 29 of gestation. These pregnancies yielded 122, 128, 109, and 132 viable foetuses, respectively. One female from the intermediate dose group (0.3 mg/kg) spontaneously aborted between days 28 - 29 of gestation.

Pregnancy rate and pre-implantation events (i.e., number of corpora lutea and implantation sites) were not effected by the administration of ribavirin (see below). There was one incident of a spontaneous abortion in a female animal from the inter-mediate dose group (0.3 mg/kg). In contrast, post-implantation fetal loss was slightly, but not significantly ($p>0.05$) increased among females in the low and intermediate dose groups as compared with the controls. Mean viable litter size was comparable for all dosage groups. However, the mean litter and foetal weights were slightly reduced among animals from the high dose treatment group as compared with the controls. The ratio of male to female foetuses was decreased (44% vs. 52%) in high dose animals.

Summary of Litter Parameters

Dose mg/kg/day	0.0	0.1	0.3	1.0
No. of Litters	15	15	13	16
Corpora Lutea	11.1	11.3	10.8	11.3
Implants	9.0	9.9	9.5	8.9
Pre-implant Loss %	19.3	11.5	12.6	19.8
Embryonic Deaths				
Early	0.4	0.9	0.5	0.4
Late	0.5	0.5	0.5	0.3
Total	0.9	1.3	1.1	0.7
Post-Implant Loss %	8.5	12.7	13.0	6.5
Live Young (Total)	122	128	109	132

Summary of Litter Parameters (Cont.)

Dose mg/kg/day	0.0	0.1	0.3	1.0
Live Young/Litter	8.1	8.5	8.4	8.3
Mean Litter Wt.	361.5	369.8	371.0	343.3
Mean Foetal Wt.	45.8	43.6	45.1	43.5

Where not specified - values are Mean/Litter

Visceral and skeletal abnormalities were evident in all groups, with slightly higher incidence in the high dose exposed foetuses. The overall incidence of foetuses from the high dose group with gross/visceral anomalies was 2-3x that of the control treated offspring. Abnormalities detected at increased frequency among the high dose exposed offspring included irregular ossification of the cervical vertebral components (18 foetuses [9 litters] from the high dose group versus 4 foetuses [3 litters] from the control group), and an increased incidence of fetuses with extra (13) thoracic-lumbar ribs. Visceral anomalies included several instances (6) of anomalous cervico-thoracic arteries in high dose exposed foetuses, although the effect did not achieve statistical significance. Variation in the frequency of foetuses with abnormal sternebrae did not suggest any drug related effects.

Comments: 1) The results suggest that ribavirin at doses between 0.1 and 0.3 mg/kg/day had no effects on weight gain, food consumption, or clinical signs in gravid female rabbits. Reductions in weight gain seen among the high dose (1.0 mg/kg) treated females during the first several days of drug administration were maintained throughout gestation, and may be related to reduced mean litter weight evident at study termination. Reductions in weight gain among the high dose treated does was probable not due to reductions in fetal survival.

2) Plasma and erythrocyte levels of ribavirin measured 1 hour following drug administration, demonstrated significant accumulation of drug with repeated dosing, and dose related increases in plasma concentrations. At 24 hours following dosing, plasma levels of drug were generally near or below the limit of detection (0.05 µM/L) in all dose groups. Erythrocyte concentrations of ribavirin typically remained elevated long after plasma levels had decreased to below the threshold of detection (confirming the accumulation and retention of ribavirin following repeated dosing).

Maximum concentrations of 0.11, 0.31, and 1.11 µM/L were measured in low, intermediate, and high dose (0.1, 0.3, and 1.0 mg/kg/day) treated animals at 1 hour following drug administration on the 18th day of gestation (11th day of drug administration).

3) While maternal toxicities were minimal, significant adverse effects on the developing embryo/foetus were evident among animals from the high dose groups. Ribavirin administered at 1 mg/kg/day, increased (2-3x) the incidence of gross visceral and skeletal abnormalities versus that observed in the controls. Abnormalities occurring in the high dose exposed foetuses included irregular ossification of the cervical vertebral components, increased incidence of fetuses with extra thoracic-lumbar ribs, and several instances of foetuses with anomalous cervico-thoracic arteries.

4) There was one incidence of an abortion in a female animal from the 0.3 mg/kg/day dose group. Abortions may occur naturally, but may also be the result of toxic drug effects. Because this abortion occurred in an animal from the intermediate dose test group, while animals in the higher dose group (1.0 mg/kg) clearly showed drug induced teratogenic effects, it is not possible to eliminate the possibility of the abortion being due to the drug treatment.

5) Within the context of the present data set, the NOEL dose for the induction of adverse teratogenic effects in the pregnant New Zealand white rabbit is estimated to be 0.1 mg/kg/day. Mean plasma levels of the test compound were generally near or below the limit of detection (0.05 µM) 1 hour following the administration of 0.1 mg/kg of ribavirin to the pregnant rabbit.

Segment III Studies

6) **Ribavirin: The Effect On The Pregnant Rat And Offspring During The Peri- and Post-Natal Period**, Study # INP 7/931920.

Status: GLP
Testing Site:
Study Initiation: 26 March 1993
Compound Tested: Ribavirin, Lot# 05500787 (R-17), purity 100%
Doses Tested: 0, 0.1, 0.3 and 1 mg/kg/day
Dose Volume and Route: 1 ml/100 g, gavage
Solvent: distilled water
Species, Strain, Sex: female Sprague-Dawley rats Crl:CD (SD) BR VAF/Plus), weight range: 182-223 grams, 25 animals/dose.

Test Procedure: Time-pregnant animals were randomly assigned to treatment groups. Drug was administered once daily by gavage, beginning on day 15 of gestation and continuing until weaning of the offspring (approximately day 22 post-partum). Animals from all drug treatment groups were allowed to give birth and rear the young to weaning.

Dose Preparations: Drug solutions were prepared each week, and were assayed for ribavirin concentration. All drug solutions showed minimal deviation from the nominal concentration.

Mortality and Clinical Signs: One dam dosed at 0.1 mg/kg/day was found dead on day 4 post-partum. The animal showed hunched posture and piloerection, and was cold to the touch, 24 hours prior to death. At necropsy, clear serous fluid was found in the abdominal cavity along with evidence of intestinal congestion. There were no other premature deaths or clinical signs noted among any of the drug treated animals.

Body Weight and Food Consumption: There were no significant effects of ribavirin treatment on the weight gain of the F_0 dams during the days prior to parturition or during lactation, or on the weight gain of their offspring (F_1 generation). Similarly, food consumption was not effected by ribavirin administration at doses of 0.1-1.0 mg/kg/day (except for an increase in food intake among animals dosed at 1.0 mg/kg/day, between days 15 and 16 post-coitus [i.e., days 1-2 of drug administration]).

Pregnancy and Litter Parameters: The mean time to parturition was equivalent for all treatment groups. All litter parameters, including: litter size, peri- and post-natal viability, litter weight, mean pup weight, sex ratio, and the time of major developmental milestones [attainment of surface and air righting reflexes, auditory startle reflex, and corneal reflex], were comparable for the treatment and control groups.

Gross Pathology: There were no drug related abnormalities noted among the F_0 dams or their offspring (F_1 generation) at the termination of the study.

Comments: 1) Ribavirin administration at doses up to 1.0 mg/kg/day was without significant adverse effects on the pregnant rats or their offspring when exposure began after the period of organogenesis and continued through weaning.

2) Failure to demonstrate any toxicity in either parent generation or offspring makes the interpretation and generalization of the study results impossible.

Appendix D: Pharmacokinetics

Summary of Pharmacokinetic Study Findings:

The results of pharmacokinetic studies conducted in the mouse, rat and dog, suggest that ribavirin when given orally either as a solution or in capsule form is well absorbed with an approximate bioavailability of 80%. The data for all 3 species, suggests that ribavirin reached maximal levels in the plasma or serum within 1-2 hours of dosing, and decayed with a half-life of between 4-10 hours. Serum and/or plasma drug levels were generally comparable for male and female animals, and for drug naive or previously drug treated animals (although slight changes in AUC values were evident in rats and dogs following repeat administration). After acute dosing, C_{max} and $AUC_{0-24\ hrs}$ values increased in a nearly linear manner, or slightly less than linear manner, with increases in the administered dose. Slight reductions in systemic exposure were evident in the rat and dog at the highest doses tested. The data suggest that the absorption of ribavirin from the gastrointestinal tract may be reduced at high doses, possibly due to saturation of a carrier mediated transport mechanism.

The sponsor has recently submitted the results of two toxicokinetics studies conducted in the mouse and rat. These studies were conducted in the same animal strains and at the same drug doses as were used in the oncogenicity studies of ribavirin. More importantly, however, was the fact that a newly developed more sensitive and more specific drug assay was used than in the previous studies (the new assay being specific to the parent drug structure versus the two primary metabolites [i.e., the deribosylated and triazole carboxamide metabolites). The results of these studies suggest that in the previously reported oncogenicity studies of ribavirin, relative interspecies 24 hour systemic drug exposure levels (at the maximum doses tested in animals versus the recommended 1200 mg clinical dose) were approximately 130% and 20% in the mouse and rat, respectively.

Tissue levels of radioactivity were nearly identical for male and female animals of each species, and were generally much higher than levels noted in the plasma and/or serum. Tissue levels of radioactivity were highest in the gastrointestinal tract, liver and kidneys (apparently related to the organs of absorption, metabolism and excretion of ribavirin). However, an exception to this was evident in the reproductive tissues of both male and female animals, which showed particularly high levels of drug following acute or repeat dose administration (the highest levels of radioactivity detected [per gram of tissue] were in the prostate of the dog). The lowest levels of radioactivity were generally detected in brain tissue.

The primary route of drug elimination was in the urine, with 50-100% of the administered radioactivity being eliminated within 24-48 hours of dosing. Approximately 5-20% of the administered radioactivity was recovered in the feces, and 10% was retained in the carcass at 24 hours after dosing (depending on the species). The entrance of ribavirin into red blood cells was somewhat delayed versus distribution of radioactivity in the plasma, suggesting that the red blood cell membrane may be semi-permeable to the passage of ribavirin and that red cells may serve as a drug reservoir (with delayed release) following drug withdrawal.

As discussed in the toxicology section of this review, ribavirin has significant adverse effects on rapidly proliferating tissues (lymphoid tissues, mucosa, spleen and testes) and those tissues with high cellular metabolism (heart, liver and secretory cells of the intestinal mucosa). The results of the pharmacokinetic and studies of ribavirin, suggest that the effected tissues are also the primary sites of drug deposition after oral

dosing.

Pharmacokinetic Studies Summary:

1) **SCH 18908: 4-Week Gavage Toxicokinetic Study In Mice**, Study ID 97347, GLP, Study Initiation: Aug. 1997, Compound Tested: SCH 18908, Lot No. 36438-027,
2) **SCH 18908: 4-Week Gavage Toxicokinetic Study In Rats**, Study ID 97348, GLP, Study Initiation: Aug. 1997, Compound Tested: SCH 18908, Lot No. 36438-027,
3) **The Disposition of Total Radioactivity In Mice Following Oral Administration of [^{14}C]-Ribavirin**, Study No. 153452, GLP, Study Initiation: 2 Apr. 1993, Ribavirin Lot# 05500787, and, [C^{14}]-Ribavirin Lot# 5382109, radiochemical purity > 99%.
4) **The Disposition of Total Radioactivity In Rats Following Oral Administration of [^{14}C]-Ribavirin**, Study No. 153468, GLP, Study Initiation: 2 Apr. 1993, Ribavirin Lot# 05500787, [^{14}C]-Ribavirin Lot# 5582109, radiochemical purity = 98.5%.
5) **Pharmacokinetics Study of Ribavirin in Dogs**, Study ID UIC/TRL No.: 110, GLP, Study Initiation: 4 Sept., 1992, Ribavirin Batch # 04200787 (D-17).
6) **The Pharmacokinetics of [^{14}C]-Ribavirin In Dogs**, Study No. 153473, GLP, Scotland, Study Initiation: 2 Apr. 1993, Ribavirin Lot# 05500787, [^{14}C]-Ribavirin Lot# 5582109, radio-chemical purity > 98.5%.
7) **The Tissue Distribution of [^{14}C]-Ribavirin In Dogs**, Study No. 153489, GLP, Study Initiation: 2 Apr. 1993, Ribavirin Lots# 05500787 and 5382109 [C^{14}], and 97%.

Pharmacokinetic Study Reviews:

1) **SCH 18908: 4-Week Gavage Toxicokinetic Study In Mice**, Study ID 97347.
Status: GLP
Study Site:
Study Initiation: Aug. 1997
Compound Tested: SCH 18908, Lot No. 36438-027,
Doses Tested: 20, 40 and 75 mg/kg/day
Route and Vol.: gavage, 15 ml/kg
Control/Solvent: Water for Inj.
Species, Sex, Age, Weight, Number: Male and Female CD-1 mice (Crl:CD-1 (ICR)BR), age approx. 6 weeks, weight range 19.1-35.8 g, 72 animals/sex/dose group.
Test Conditions: Groups of animals were dosed with ribavirin by oral gavage, once per day for 1 and 29 days. Animals were sacrificed at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours following dosing (3 animals/sex/dose/time point). Pooled serum samples were used for the assessment of drug levels. Mortality, morbidity and clinical signs were monitored daily, while body weight was monitored once per week.
Results: There were no apparent drug related effects on body weight, clinical signs, morbidity or mortality at any dose tested.
Dose Solutions: All drug dosing solutions were assayed as within 5% of nominal concentration.
Pharmacokinetic Parameters: There were no differences detected in the pharmacokinetic parameters for male and female animals at any of the doses tested. Therefore, the following table presents the combined pharmacokinetic

values for male and female animals dosed with SCH 18908 (ribavirin).

Single and Repeat Dose Pharmacokinetic Values in Mice (Male and Female data combined) Following Oral Administration

Dose mg/kg	20	40	75
Day 1			
Cmax (ng/ml)	3600	4160	6440
Tmax (hr)	1	1	0.5
AUC (ng.hr/ml)	13500	23800	43800
t1/2 (hr)	4.6	6.6	5.2
Day 29			
Cmax (ng/ml)	2970	4360	5900
Tmax (hr)	0.25	0.5	0.5
AUC (ng.hr/ml)	13900	23500	38000
t1/2 (hr)	10.4	6.5	7.1

Comments: 1) This study was conducted using a newly developed assay for ribavirin.

2) Cmax and AUC values for unmetabolised ribavirin represent approximately 10-20% of the values previously estimated by radioisotopic tracer methods. A similar ratio of metabolized and unmetabolised drug product was seen in human subjects during pharmacokinetic trials conducted with ribavirin (see BioPharmacology Review).

3) The drug doses tested in this study were the same as those utilized in the 18 month mouse oncogenicity study. The results of this study suggest that in the previous oncogenicity study the maximum 24 hour systemic exposure (AUC 24 hr) to ribavirin was approximately 130% of the 24 hour systemic exposure to drug as obtained in human subjects receiving 1200 mg ribavirin per day.

4) Cmax and AUC values increased in a slightly less than proportional manner with increase in the oral dose of ribavirin to male and female mice.

5) Pharmacokinetic parameters were similar in male and female mice following oral administration at doses of 20-75 mg/kg/day.

6) No evidence of drug accumulation in the blood plasma compartment was evident during the 29 days of repeat-dose oral administration.

7) The absorption and elimination kinetics of ribavirin were dose-independent in male and female mice dosed at 20-75 mg/kg. The systemic absorption of ribavirin following oral administration in mice was rapid, reaching maximal serum drug concentrations at 0.25-1 hour after drug administration. The terminal elimination half-time of ribavirin from the plasma drug compartment was

between 5.2 and 10.4 hours.

2) **SCH 18908: 4-Week Gavage Toxicokinetic Study In Rats,** Study ID 97348.
Status: GLP
Study Site:
Study Initiation: Aug. 1997
Compound Tested: SCH 18908, Lot No. 36438-027, 101% Pure.
Doses Tested: 10, 20 and 40 mg/kg/day
Route and Vol.: gavage, 5 ml/kg
Control/Solvent: Water for Inj.
Species, Sex, Age, Weight, Number: Male and Female Sprague-Dawly rats (Crl:CD (SD)BR), age 7-8 weeks, weight range 183 - 426 g, 72 animals/sex/dose group.
Test Conditions: Groups of animals were dosed with ribavirin by oral gavage, once per day for 1 and 29 days. Animals were sacrificed at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours following dosing (3 animals/sex/dose/time point). Mortality, morbidity and clinical signs were monitored daily, while body weight was monitored once per week.
Results: There were no apparent drug related effects on body weight,. clinical signs, morbidity or mortality at any dose tested.
Dose Solutions: All drug dosing solutions were assayed as within 5% of nominal concentration.
Pharmacokinetic Parameters: The following table presents the pharmacokinetic values for male and female rats dosed with ribavirin (oral) for 1 and 29 days.

Single and Repeat Dose Pharmacokinetic Values in Rats Following Oral Administration

Dose mg/kg	10		20		40	
	♂	♀	♂	♀	♂	♀
Day 1						
Cmax	193	127	372	222	565	383
Tmax	1.5	1.5	1.5	1.5	1.5	1.0
AUC	566	276	1500	760	4690	3240
t1/2	ND	ND	ND	ND	5.2	ND
Day 29						
Cmax	181	160	305	241	435	437
Tmax	1	0.5	4	2	3	0.5
AUC	989	441	3210	1620	5540	4830
t1/2	ND	ND	ND	ND	5.1	ND

ND - Value not computed due to insufficient sample numbers.

Comments: 1) This study was conducted using a newly developed assay for ribavirin.

2) Cmax and AUC values for unmetabolised ribavirin represent approximately 50 and 10% of the values previously estimated by radioisotopic tracer methods. A similar ratio of metabolized and unmetabolised drug product was seen in human subjects during pharmacokinetic trials with ribavirin (see BioPharm. Review).

3) The drug doses tested in this study were the same as those

utilized in the 24 month rat oncogenicity study. The results of this study suggest that in the previous oncogenicity study the maximum 24 hour systemic exposure (AUC 24 hr) to ribavirin was approximately 20% of the 24 hour systemic exposure to drug as obtained in human subjects receiving 1200 mg ribavirin per day.

4) Cmax values increased in a less than dose proportional manner in both male and female rats dosed orally with ribavirin at 10-40 mg/kg. In contrast, 24 hour AUC values increased in a more than dose proportional manner among both male and female rats. Similar effects on Cmax and AUC were observed following acute (day 1) and repeat dosing (day 29) with ribavirin.

5) Peak (Cmax) and total systemic (AUC) exposure to ribavirin in male rats was approximately 1.5-2x that observed in female rats at each dose tested.

6) AUC values following repeat drug administration (day 29) were approximately 1.25-2x those observed following acute drug administration (day 1) in both male and female rats. The study results suggest that ribavirin was accumulating in the plasma drug compartment following once/day dosing at 10-40 mg/kg/day.

3) **The Disposition of Total Radioactivity In Mice Following Oral Administration of [C^{14}]-Ribavirin**, Study No. 153452.

Status: GLP
Study Site:
Study Initiation: 2 Apr. 1993
Compound Tested: Ribavirin Lot# 05500787, and [C^{14}]-Ribavirin Lot# 5382109, radiochemical purity $\geq$ 99%.
Doses Tested: 18.75, 37.5, 75 and 150 mg/kg
Dose Volume and Route: 10 ml/kg, oral gavage
Solvent: water
Species, Strain, Sex: male & female CD-1 mice, age 4-5 weeks, 32 animals/sex/dose.
Test conditions: Radiolabelled ribavirin was administered on day 1 or 30 of treatment. Animals treated with [C^{14}]-ribavirin on Day 30, received unlabelled ribavirin once/day for the preceding 29 days. Radioactivity was measured in the plasma, red blood cells and excreta at intervals up to 24 hours, and in tissues at 2 hours after dosing. The study results are summarized below.

Mean [C^{14}]-Ribavirin Concentration in Selected Tissues At 2 Hours Post-Administration In The Male SD Rat

Dose (mg/kg)		18.75	37.5	75	150
Brain	♂	1.37	2.45	3.96	5.08
	♀	1.41	1.71	3.55	6.31
Kidney	♂	18.45	33.63	55.31	78.08
	♀	15.41	19.52	43.91	90.54
Liver	♂	66.83	163.37	274.95	350.16
	♀	62.71	126.37	280.27	503.82
Heart	♂	19.26	31.14	55.93	68.89
	♀	23.14	27.12	63.25	98.17
Lymph Nodes	♂	29.19	36.38	56.15	87.86
	♀	26.38	20.36	41.53	90.49
Skeletal Muscle	♂	7.58	14.65	22.70	27.35
	♀	7.45	12.09	24.17	36.36
Plasma (ml)	♂	2.61	8.10	12.57	18.79
	♀	3.32	5.63	10.12	22.02

Values are in µMoles/gram

Summary of Ribavirin Plasma PK in the CD-1 Mouse

Dose mg/kg	Sex	C_{max} µg/ml	T_{max} hr	$t_{1/2}$ hr	AUC_{0-24hr} µg.hr/ml
Day 1					
18.75	♂	4.83	0.25	5.77	29.22
	♀	5.56	0.5	5.0	30.35
37.5	♂	8.28	1.0	6.67	53.20
	♀	9.22	1.0	5.94	47.75
75.0	♂	13.53	0.5	8.35	108.11
	♀	12.58	0.5	5.79	97.30
150	♂	20.61	1.0	9.66	225.52
	♀	22.02	2.0	6.05	204.64
Day 30					
18.75	♂	7.32	0.5	4.5	29.55
	♀	5.57	0.25	4.67	27.14
37.5	♂	9.63	0.5	9.03	60.73
	♀	11.77	0.5	5.37	53.58
75.0	♂	12.80	0.5	6.66	104.07
	♀	9.32	1.0	10.77	78.79
150	♂	18.27	1.0	11.23	148.45
	♀	22.84	2.0	7.10	142.77

(µg/gr = µg [C^{14}]-ribavirin equivalent/gr)

Comments: 1) The data contained in the preceding tables indicates that levels of ribavirin (measured as radioactive equivalents) generally reached maximal levels within 1-2 hours of oral dosing, and decayed with a half-life of between 4-10 hours.

2) Plasma drug levels (radioactivity) were similar for male and female animals, and for drug naive (day 1) or previously treated animals (day 30). Although a slight decrease in the 24 hour AUC value was evident for male and female animals dosed at 150 mg/kg, when comparing the value on day 30 of drug administration with the value for day 1. No obvious explanation for the change in AUC is evident, as all other parameters were similar for the 2 test dates.

3) Comparison of the C_{max} and $AUC_{0-24\ hrs}$ data for total systemic exposure to radioactivity suggests that the exposure increased in a nearly linear manner at doses up to 37.5 mg/kg, but then decreased with further increases in the administered dose. The data suggest that at doses above 37.5 mg/kg, the absorption of ribavirin from the gastrointestinal tract was reduced, possibly due to the saturation of a carrier transport.

4) Although not shown in the preceding tables, the study results suggest that the entrance of ribavirin into red cells is somewhat delayed versus distribution of radioactivity in the plasma. As in most other species, the levels of radioactivity within red blood cells was approximately 2-4x that seen in the plasma 24 hours following drug administration. These data suggest that red blood cells may serve as a drug reservoir with delayed release.

5) Tissue levels of radioactivity were nearly identical for male and female animals, and were generally much higher than levels noted in the plasma. Tissue levels of radioactivity were highest in the gastrointestinal tract, liver and kidneys, and appeared related to the major organs of absorption, metabolism and excretion of ribavirin. The lowest levels of radioactivity were

generally detected in brain tissue.

6) The primary route of drug elimination was in the urine, with 50-80% of the administered radioactivity being eliminated within 24 hours of dosing. Approximately 6-16% of the administered radioactivity was recovered in the feces, and 10% was retained in the carcass at 24 hours after dosing. The recovery of radioactivity after dosing at 150 mg/kg differed somewhat from the lower doses, with a larger proportion of the radioactivity being recovered in the feces (mean fecal recovery of 45% and urinary drug recovery of 30%). The recovery of radioactivity during the 24 hours after dosing was slightly less than quantitative at approximately 90-95% of the administered dose.

4) **The Disposition of Total Radioactivity In Rats Following Oral Administration of [^{14}C]-Ribavirin**, Study No. 153468.

Status: GLP
Study Site:
Study Initiation: 2 Apr. 1993
Compound Tested: Ribavirin Lot# 05500787, purity > 99% and [^{14}C]-Ribavirin Lot# 5582109, radiochemical purity = 98.5%.
Doses Tested: 0.3, 1.5, 7.5 and 40 mg/kg
Dose Volume and Route: 4 ml/kg, oral gavage
Solvent: water
Species, Strain, Sex: male & female SD rats, age 7-8 weeks, 8 animals/sex/dose.
Test conditions: Radiolabelled ribavirin was administered on day 1 or 30 of treatment. Animals treated with [^{14}C]-ribavirin on Day 30, received unlabelled ribavirin once/day for the preceding 29 days. Radioactivity was measured in the plasma, red blood cells and excreta at intervals up to 24 hours, and in tissues at 2 hours after dosing. The study results are summarized below.

Summary of Ribavirin Plasma PK in the SD Rat

Dose mg/kg	Sex	C_{max} µg/ml	T_{max} hr	$t_{1/2}$ hr	AUC_{0-24hr} µg.hr/ml
Day 1					
0.3	♂	.066	1	5	0.44
	♀	.086	2	7	0.43
1.5	♂	0.43	2	5	2.60
	♀	0.48	2	5	2.46
7.5	♂	1.89	2	5	13.05
	♀	2.22	2	5	12.88
40	♂	10.9	2	5	76.25
	♀	8.10	2	4	69.10
Day 30					
0.3	♂	.087	1	6	0.54
	♀	.099	1	5	0.56
1.5	♂	0.43	2	5	2.39
	♀	0.48	1	5	2.85
7.5	♂	2.47	2	7	13.90
	♀	2.36	2	5	13.78
40	♂	6.40	2	6	69.41
	♀	8.5	2	5	79.13

(µg/ml = µg [^{14}C]-ribavirin equivalents/ml)

Summary of Ribavirin Red Blood Cell PK in the SD Rat

Dose mg/kg	Sex	C_{max} µg/gr	T_{max} hr	$C_{24\ hr}$ µg/gr	Cell/Plasma Ratio T_{max}	Cell/Plasma Ratio 24 hrs
Day 1						
0.3	♂	.069	2	.008	1.3	3.8
	♀	.098	2	.008	1.2	5.8
1.5	♂	0.45	2	0.04	1.1	4.0
	♀	0.51	2	0.03	1.1	3.5
7.5	♂	1.90	2	0.15	1.0	2.8
	♀	2.33	2	0.13	1.1	2.7
40	♂	10.4	2	0.8	1.0	2.6
	♀	8.4	2	0.9	1.1	2.9
Day 30						
0.3	♂	.095	1	.012	1.2	4.9
	♀	.105	1	.011	1.1	5.1
1.5	♂	0.44	2	0.06	1.0	6.0
	♀	0.48	2	0.06	1.0	4.8
7.5	♂	2.38	2	0.16	1.0	2.8
	♀	2.29	2	0.24	1.0	4.8
40	♂	7.0	2	1.3	1.1	2.4
	♀	8.1	2	1.4	1.0	3.3

(µg/g = µg [^{14}C]-ribavirin equivalents/g)

Mean [^{14}C]-Ribavirin Concentration in Selected Tissues At 2 Hours Post-Administration In The Male SD Rat

Dose (mg/kg)	0.3	1.5	7.5	40
Brain	.042	.18	.95	2.4
Kidney	.463	3.94	11.63	32.4
Liver	.678	3.59	21.13	90.6
Skeletal Muscle	.109	.52	2.78	6.5
Plasma (ml)	.082	.41	2.47	6.4

Values are in µMoles [^{14}C]-ribavirin equivalents/gram of tissue

Comments: 1) Data contained in the preceding tables indicates that levels of radioactivity (and by inference the concentration of ribavirin) reached maximal levels within 1-2 hours of oral dosing, and decayed with a half-life of between 4-8 hours. Furthermore, the plasma drug levels (radioactivity) were comparable for male and female animals, and for drug naive (day 1) or previously treated animals (day 30).

2) Comparison of the Cmax and AUC data for total systemic exposure to radioactivity suggests that the exposure increased in a nearly linear manner with increases in the administered dose.

3) Comparison of the kinetic parameters for radio-activity in plasma and red blood cells, suggests that the entrance of ribavirin into red cells is somewhat delayed versus distribution of radioactivity in the plasma. Twenty-four hours following drug dosing, the levels of radioactivity within red blood cells was approximately 2-4x that seen in the plasma, suggesting that the cells may serve as a drug reservoir with delayed release.

4) Tissue levels of radioactivity were nearly identical for male and female animals, and were generally higher than levels noted in the plasma. Tissue levels of radioactivity were highest in the

gastrointestinal tract, liver and kidneys, and appeared related to the major organs of absorption, metabolism and excretion of ribavirin. The lowest levels of radioactivity were generally detected in brain tissue.

5) The primary route of drug elimination was in the urine, with 71-83% of the administered radioactivity being eliminated within 24 hours of dosing. Approximately 5-11% of the administered radioactivity was recovered in the feces, while 10-14% was retained in the carcass at 24 hours after dosing. Total recovery of radioactivity during the 24 hours after dosing ranged from 99% to 105% of the administered dose.

5) **Pharmacokinetics Study of Ribavirin in Dogs**, Study ID UIC/TRL No.: 110.
Status: GLP
Study Initiation: 4 Sept., 1992
Study Site:

Compound Tested: Ribavirin, Batch # 04200787 (D-17)
Dose(s) Tested: 30 mg/kg
Dose Volume and Route: 1 ml/kg gavage or gelatin capsule, oral
Solvent and Control: distilled H_2O or gelatin capsule
Species, Strain, Sex: Male beagle dogs, age 6.5-7 months at study initiation, weight range 10.1-11.4 kg.
Test Conditions: Animals were randomly allocated into 2 groups of 3 animals each. A single oral administration of test compound was performed by gastric (gavage) or oro-tracheal (capsule) intubation. Blood samples were drawn at 0, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 and 196 hours following ribavirin dosing for the determination of plasma drug levels. Clinical signs and mortality were measured periodically during the post-dosing interval. No gross or microscopic evaluations were performed on the study animals.

There were no premature deaths among the study animals. Clinical signs were not affected by the administration of ribavirin in either formulation.

Generally, the data indicate that the kinetics of ribavirin were independent of the drug formulation. The only exception to this was related to the plasma elimination half-time ($t_{1/2,\ elimination}$) which was somewhat shorter following capsule administration then after the gavage treatment. Ranges of and hrs. were seen following capsule and gavage treatment, respectively. This effect appeared related to a more rapid absorption phase in 2/3 capsule treated animals as compared with the gavage treated animals (in which 1/3 animals showed increased absorption). The absorption half-time (in the previously mentioned 3 animals) was approximately 0.5 hrs.

Peak plasma levels were seen in all animals within the first 2 hours following gavage or capsule administration (mean 1.4 hrs.), and ranged from µM/l (mean 29.3 µM/l). The mean $AUC^{(0-196\ hrs.)}$ following the 30 mg/kg dose was 335.5 µM.hrs/l and appeared independent of the drug formulation. Drug clearance from plasma was estimated at 4.4 l/hr, with a volume of distribution of approximately 5x total body water.

Comments: 1) The results of the present study suggest that the pharmacokinetics of ribavirin are comparable following oral capsule or solution (gavage) administration in the male beagle dog.

2) The study results may be best described by one- or two-compartment open models, the best fit being dependent upon the rapidity of the early absorption phase for ribavirin (one-

compartment model without rapid absorption phase, versus a two-compartment model when rapid absorption is evident).

6) **The Pharmacokinetics of [^{14}C]-Ribavirin In Dogs**, Study No. 153473.
Status: GLP
Study Site:
Study Initiation: 2 Apr. 1993
Compound Tested: Ribavirin Lot# 05500787, and
[^{14}C]-Ribavirin Lot# 5582109, radiochemical purity = 98.5%.
Doses Tested: 5.0 and 20.0 mg/kg
Dose Volume and Route: 110 ml, oral gavage
Solvent: deionized water
Species, Strain, Sex: male & female Beagle dogs, weight range 5.9-11.1 kg, 3 animals/sex/dose.
Test conditions: Radiolabelled ribavirin was administered to each animal on 2 occasions (Days 1 and 30 of dosing). On all intervening test days, non-radiolabelled ribavirin was administered. Following drug administration, total radioactivity was measured in the plasma, red blood cells and excreta collected at intervals up to 48 hours.

The following table presents summary pharmacokinetic data for ribavirin when administered orally to male and female dogs.

Pharmacokinetic Parameters for Plasma Ribavirin in the Beagle Dog

Dose (mg/kg)	5.0		20.0	
Parameter	♂	♀	♂	♀
Day 1				
C_{max} (µg/ml)	2.3	2.1	8.4	10.5
T_{max} (hrs)	1.0	0.5	0.5	0.3
$t_{1/2}$ (hrs)	8.7	8.8	8.8	9.5
AUC (µg/ml/hr)	18.9	18.6	77.0	74.3
Day 30				
C_{max} (µg/ml)	2.5	2.7	9.4	8.2
T_{max} (hrs)	0.3	0.3	0.3	0.5
$t_{1/2}$ (hrs)	8.3	9.6	9.6	11.7
AUC (µg/ml/hr)	21.6	20.8	90.9	95.2

(µg/ml = µg [^{14}C]-ribavirin equivalent/ml)

Comments: 1) The study results suggest that ribavirin was rapidly absorbed from the gastrointestinal tract following oral administration, with maximal drug levels (as measured by total radioactivity) being reached in $\leq$ 1 hour. Plasma drug levels were similar for male and female animals, and following the initial (day 1) or 30th dose of drug.

2) Systemic exposure to radioactivity (ribavirin), whether measured by Cmax or AUC, increased in a nearly linear manner with increases in the administered dose.

3) As noted in the SD rat, the concentration of radioactivity (ribavirin) in red blood cells lags behind the levels seen in the blood during the initial 2-4 hours after drug administration. Thereafter, the concentration of radioactivity in the cells exceeds the levels detected in the plasma. The data suggest that the red cell membrane may act as a semi-permeable barrier to the passage of ribavirin (either into or out of the cell), and that the red blood cells may act as a drug reservoir (with delayed release) which may maintain systemic drug exposure long after

dosing has been discontinued.

4) Approximately 82-97% of the administered radioactivity was recovered in the urine of male and female dogs within 48 hours of dosing. An additional 2-3% of the administered radioactivity was recovered in the feces during the same time interval. No assessment of radioactivity in individual tissues or the total carcass was performed. Total recovery of radioactivity during the 48 hours after dosing ranged of the administered dose.

7) **The Tissue Distribution of [C^{14}]-Ribavirin In Dogs,** Study No. 153489.
Status: GLP
Study Site:
Study Initiation: 2 Apr. 1993
Compound Tested: Ribavirin Lot# 05500787, and
[C^{14}]-Ribavirin Lot# 5382109, radiochemical purity $\geq$ 97%.
Doses Tested: 5.0 and 20.0 mg/kg
Dose Volume and Route: 80 ml, oral gavage
Solvent: deionized water
Species, Strain, Sex: male & female Beagle dogs, weight range 5.9-11.1 kg, 2 animals/sex/dose.
Test conditions: Radiolabelled ribavirin was administered to animals on 1 of 2 occasions (Days 1 or 18 of dosing). Nonlabelled ribavirin was administered on days 1-17. Tissue specimens were collected at 24 following drug administration for the determination of total radioactivity.

The table on the following page presents summary data for tissue levels of ribavirin administered orally to male and female beagle dogs.

Tissue [C^{14}]-Ribavirin Levels in the Beagle Dog

Dose (mg/kg)	5.0		20.0	
Parameter	♂	♀	♂	♀
Values are in µg [C^{14}]-ribavirin equivalent/gram of tissue				
Day 1				
Adrenals	1.26	0.80	3.18	5.78
Brain	0.44	0.41	1.40	1.76
Heart	0.34	0.28	0.99	1.44
Intestine Wall				
Large	0.70	0.67	2.21	2.99
Small	0.94	0.82	2.69	3.92
Kidney	1.82	1.46	5.51	10.20
Liver	2.54	1.94	5.35	20.02
Lung	0.95	0.71	2.10	4.91
Lymph Node				
Mesenteric	1.32	0.98	3.33	7.00
Muscle	0.41	0.38	1.25	1.80
Ovaries	----	0.79	----	2.33
Prostate	3.55	----	5.64	----
Skin	0.41	0.35	1.55	2.16
Stomach Wall	0.48	0.45	1.49	2.38
Testes	1.31	----	4.16	----
Uterus	----	1.30	----	3.48
Plasma (ml)	0.22	0.22	0.66	1.17
Red Blood Cells	0.34	0.34	1.11	1.35
Day 18				
Adrenals	1.14	1.44	6.82	5.52
Brain	0.48	0.52	2.19	1.91
Heart	0.37	0.39	1.86	1.80

Tissue [C^{14}]-Ribavirin Levels in the Beagle Dog (Cont.)

Dose (mg/kg)	5.0		20.0	
Parameter	♂	♀	♂	♀
Values are in µg [C^{14}]-ribavirin equivalent/gram of tissue				
Intestine Wall				
Large	0.67	0.71	4.42	3.22
Small	1.11	1.01	4.35	5.03
Kidney	1.88	2.44	11.20	9.96
Liver	2.15	2.78	15.08	14.74
Lung	0.82	1.32	4.89	3.70
Lymph Node				
Mesenteric	1.28	0.77	5.71	3.42
Muscle	0.48	0.49	3.56	2.42
Ovaries	----	0.64	----	2.53
Prostate	2.93	----	24.61	----
Skin	0.65	0.76	2.29	2.29
Stomach Wall	0.58	0.64	3.70	2.88
Testes	0.95	----	4.57	----
Uterus	----	1.12	----	3.72
Plasma (ml)	0.27	0.28	1.37	1.45
Red Blood Cells	0.32	0.35	1.30	1.59

Comments: 1) The study results suggest that tissue drug levels were nearly comparable for male and female animals following the administration of [C^{14}] radiolabelled ribavirin on day 1 or 18 of testing. Tissue drug levels generally exceeded the plasma concentration of radio-activity. Brain tissue consistently showed lower levels of radioactivity than were detected in the plasma.

2) Tissue levels of radioactivity were generally highest in the tissues/organs responsible for the elimination of metabolism of ribavirin (i.e., kidney and liver, but were also high in mesenteric lymph node, adrenal glands, lungs and the intestinal wall.

3) The reproductive tissues of both male and female animals showed particularly high concentrations of radioactivity following either single or repeat dose administration of ribavirin. The highest levels of radioactivity detected (per gram of tissue) were in the prostate.

4) For the majority of tissues, the concentration of radioactivity detected following dosing at 20 mg/kg was 3-5x the levels detected at a dose of 5 mg/kg. The data suggest that the absorption/distribution/elimination of ribavirin is linearly dose-proportional at doses of 20 mg/kg or less (in the beagle dog). Exceptions to this pattern included the prostate gland and liver (males and females) which showed greater than proportional increases in the tissue levels of radioactivity following dosing at 20 mg/kg (versus 5 mg/kg).

Appendix E: Toxicology Sections of the INTRON A/REBETOL Label

Summary of Product Label Review:

The sponsors proposed new product label for INTRON A in combination with REBETOL, as relates to the carcinogenic/mutagenic, teratogenic and reproductive effects of each drug, has been reviewed in this document. Revisions to the proposed labelling have been made and communicated to the sponsor by letter on 1 and 5 May 1998. Portions of the proposed text of the final product label, as pertains to the non-clinical safety sections of the product label, are presented on the following pages under the heading of "FDA Proposed Label Revisions."

Product Label Review:

SPONSOR'S PROPOSED LABEL
(As submitted 1 May 1998)

from **Boxed Warning:**

Combination INTRON A/REBETOL therapy is contraindicated in women who are pregnant. Women of childbearing potential and men must use effective contraception during treatment and during the 6 month posttreatment follow up period. Significant teratogenic and/or embryocidal potential has been demonstrated for ribavirin in all animal species studied. See CONTRAINDICATIONS.

from **CONTRAINDICATIONS:**

Carcinogenesis, Mutagenesis, Impairment of Fertility: Studies with interferon alfa-2b, recombinant have not been performed to determine carcinogenicity. Valid chronic carcinogenicity studies cannot be performed because neutralizing activity appears in the serum after multiple dosing in all of the animal species tested.

Adequate studies to assess the carcinogenic potential of ribavirin in animals have not been conducted. However, ribavirin is a nucleoside analog that has produced positive findings in in vitro and animal in vivo genetic toxicity assays, and could be considered a potential carcinogen. Further studies to assess the carcinogenic potential of ribavirin in animals are ongoing.

No reproductive toxicology studies have been performed using interferon alfa-2b, recombinant in combination with ribavirin. Evidence provided below for interferon alfa-2b, recombinant or ribavirin alone indicate that both agents have effects on reproduction. It can be reasonably assumed that the effects produced by either agent alone would also be caused by the combination of the two agents. Interferons may impair fertility. In studies of interferon alfa-2b administration in nonhuman primates, menstrual cycle abnormalities have been observed. Decreases in serum estradiol and progesterone concentrations have been reported in women treated with human leukocyte interferon.

In addition, ribavirin is embryotoxic and/or teratogenic at doses well below the recommended human dose in all animal species in which studies have been conducted. Therefore, fertile women should not receive combination INTRON A/ REBETOL therapy unless they are using effective contraception during the therapy period. Based on a multiple dose $t_{1/2}$ of ribavirin of 12 days, effective contraception should be utilized for 6 months post therapy (e.g., 15

half-lives of clearance for ribavirin).

Combination INTRON A/REBETOL therapy should be used with caution in fertile men. In a study in mice to evaluate time course and reversibility of ribavirin-induced testicular degeneration at doses of 35 to 150 mg/kg/day (estimated human equivalent of 2.92 - 12.5 mg/kg/day, based on body surface area adjustment for the adult) administered for three or six months, abnormalities in sperm occurred. Upon cessation of treatment, essentially total recovery from ribavirin-induced testicular toxicity was apparent within one or two spermatogenesis cycles. A follow up study to further assess these findings is ongoing.

Mutagenicity studies have demonstrated that interferon alfa-2b, recombinant is not mutagenic. Mutagenicity studies suggest that ribavirin may exert some mutagenic activity. Ribavirin is considered to be active in the Balb/3T3 In Vitro Transformation Assay. Mutagenic activity was observed in the mouse lymphoma assay, and at doses of 20-200 mg/kg (estimated human equivalent of 1.67 - 16.7 mg/kg, based on body surface area adjustment for a 60 kg adult) in a mouse micronucleus assay. A dominant lethal assay in rats was negative, indicating that if mutations occurred in rats they were not transmitted through male gametes.

Animal Toxicology: Long term studies in the mouse and rat (18 - 24 months; doses of 20 - 75 and 10 - 40 mg/kg/day, respectively {estimated human equivalent of 1.67 - 6.25 and 1.43 - 5.71 mg/kg/day, respectively, based on body surface area adjustment for the adult}) have demonstrated a relationship between chronic ribavirin exposure and increased incidence of vascular lesions (microscopic hemorrhages) in mice. In rats, retinal degeneration occurred in controls, but the incidence was increased in ribavirin-treated rats.

Pregnancy Category X (see CONTRAINDICATIONS.): Interferon alfa-2b, recombinant has been shown to have abortifacient effects in Macaca mulatta (rhesus monkeys) at 7.5, 15, and 30 million IU/kg (estimated human equivalent of 2.5, 5, and 10 million IU/kg, based on body surface area adjustment for the adult). Although abortion was observed in all dose groups, it was only statistically significant at the mid- and high-dose groups. There are no adequate and well-controlled studies in pregnant women.

Ribavirin was embryotoxic and/or teratogenic in conventional embryotoxicity/ teratogenicity studies in rats and rabbits at dose levels well below those proposed for clinical use. A no-effect dose level of 0.3 mg/kg/day for both the rat and rabbit produced ribavirin concentrations in plasma/erythrocytes of <0.06/1.51 (rat) and 0.31/4.12 (rabbit) TM/L. No maternal toxicity nor effects on offspring were observed in a peri/postnatal toxicity study with rats orally dosed with levels as high as 1 mg/kg/day.

Women of childbearing potential should not receive combination INTRON A/REBETOL therapy unless they are using effective contraception during the therapy period. In addition, effective contraception should be utilized for 6 months post therapy based on a multiple dose $t_{1/2}$ of ribavirin of 12 days.

Exposure of Female Partners: Potential Risk to Fetus Ribavirin is known to accumulate in intracellular components from where it is cleared very slowly. It is not known whether ribavirin contained in sperm will exert its known teratogenic effects upon fertilization of the ova. In a dominant lethal study in rats, it was concluded that dominant lethality was not induced by ribavirin at doses up to 200 mg/kg for 5 days (estimated human equivalent of 7.14 - 28.6 mg/kg, based on body surface area adjustment for the adult). However, because of the known teratogenic effects of ribavirin exposure to the fetus, male patients should be advised to take every precaution to avoid risk of pregnancy for their female partners.

It is advised that male patients be counseled to practice effective contraception during treatment with combination INTRON A/REBETOL therapy and for the 6 month post therapy period (e.g., 15 half-lives for ribavirin clearance from the body).

FDA PROPOSED LABEL REVISIONS
(As communicated to the Sponsor on 1 and 5 May 1998.)

To be included in **BOXED WARNING:**

Combination INTRON A®/REBETOL® therapy is contraindicated in women who are pregnant. Women of childbearing potential and men must use effective contraception during treatment and during the 6 month posttreatment follow up period. Significant teratogenic and/or embryocidal potential has been demonstrated for ribavirin in all animal species studied. It should be assumed that combination INTRON A® and REBETOL® may cause fetal harm in humans. (See CONTRAINDICATIONS.)

To be included in **CONTRAINDICATIONS:**

INTRON A® Injection in combination with REBETOL® Capsules is contraindicated in patients with a history of hypersensitivity to alpha interferons and/or ribavirin or any component of the injection and/or capsule.

Combination INTRON A®/REBETOL® therapy must not be used by women who are or may become pregnant. Women of childbearing potential and men must use effective contraception during treatment and during the 6 month post-treatment follow up period. Significant teratogenic and/or embryocidal potential has been demonstrated for ribavirin in all animal species in which adequate studies have been conducted. These effects occurred at doses as low as one twentieth of the recommended human dose of REBETOL®. In addition, interferon demonstrated abortifacient effects when administered to non-human primates. Therefore, although clinical studies have not been performed, it should be assumed that REBETOL® and/or INTRON A® may cause fetal harm in humans.

Carcinogenesis and Mutagenesis: Carcinogenicity studies with interferon alfa-2b, recombinant have not been performed, because neutralizing activity appears in the serum after multiple dosing in all of the animal species tested.

Adequate studies to assess the carcinogenic potential of ribavirin in animals have not been conducted. However, ribavirin is a nucleoside analog that has produced positive findings in multiple *in vitro* and animal *in vivo* genotoxicity assays, and should be considered a potential carcinogen. Further studies to assess the carcinogenic potential of ribavirin in animals are ongoing.

Mutagenicity studies have demonstrated that interferon alfa-2b, recombinant is not mutagenic. Ribavirin demonstrated increased incidence of mutation and cell transformation in multiple genotoxicity assays. Ribavirin was active in the Balb/3T3 *In Vitro* Cell Transformation Assay. Mutagenic activity was observed in the mouse lymphoma assay, and at doses of 20-200 mg/kg (estimated human equivalent of 1.67 - 16.7 mg/kg, based on body surface area adjustment for a 60 kg adult; 0.1-1.0X the maximum recommended human 24 hour dose of ribavirin) in a mouse micronucleus assay. A dominant lethal assay in rats was negative, indicating that if mutations occurred in rats they were not transmitted through male gametes.

Impairment of Fertility: No reproductive toxicology studies have been performed using interferon alfa-2b, recombinant in combination with ribavirin. However, evidence provided below for interferon alfa-2b, recombinant and ribavirin when administered alone indicate that both agents have adverse effects on reproduction. It should be assumed that the effects produced by either agent alone will also be caused by the combination of the two agents. Interferons may impair human fertility. In studies of interferon alfa-2b recombinant administration in nonhuman primates, menstrual cycle abnormalities have been observed. Decreases in serum estradiol and progesterone concentrations have been reported in women treated with human leukocyte interferon. In addition, ribavirin demonstrated significant embryotoxic and/or teratogenic

effects at doses well below the recommended human dose in all animal species in which adequate studies have been conducted.

Fertile women should not receive combination INTRON A®/ REBETOL® therapy unless they are using effective contraception during the therapy period. Based on a multiple dose $t_{1/2}$ of ribavirin of 12 days, effective contraception should be utilized for 6 months post therapy (e.g., 15 half-lives of clearance for ribavirin).

Combination INTRON A®/REBETOL® therapy should be used with caution in fertile men. In a study in mice to evaluate the time course and reversibility of ribavirin-induced testicular degeneration at doses of 35 to 150 mg/kg/day (estimated human equivalent of 2.92 - 12.5 mg/kg/day, based on body surface area adjustment for a 60 kg adult; 0.2 - 0.8X the maximum recommended human 24 hour dose of ribavirin) administered for three or six months, abnormalities in sperm occurred. Upon cessation of treatment, essentially total recovery from ribavirin-induced testicular toxicity was apparent within one or two spermatogenesis cycles. A follow up study to further assess these findings is ongoing.

Animal Toxicology: Long term studies in the mouse and rat (18 - 24 months; doses of 20 - 75 and 10 - 40 mg/kg/day, respectively (estimated human equivalent doses of 1.67 - 6.25 and 1.43 - 5.71 mg/kg/day, respectively, based on body surface area adjustment for a 60 kg adult; approximately 0.1-0.4X the maximum human 24 hour dose of ribavirin)) have demonstrated a relationship between chronic ribavirin exposure and increased incidence of vascular lesions (microscopic hemorrhages) in mice. In rats, retinal degeneration occurred in controls, but the incidence was increased in ribavirin-treated rats.

Pregnancy Category X (see CONTRAINDICATIONS.): Interferon alfa-2b, recombinant has been shown to have abortifacient effects in *Macaca mulatta* (rhesus monkeys) at 15 and 30 million IU/kg (estimated human equivalent doses of 5 and 10 million IU/kg, based on body surface area adjustment for a 60 kg adult). There are no adequate and well-controlled studies in pregnant women.

Ribavirin produced significant embryotoxic and/or teratogenic effects in all animal species in which adequate studies have been conducted. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were noted. The incidence and severity of teratogenic effects increased with escalation of the drug dose. Survival of fetuses and offspring was reduced. In conventional embryotoxicity/teratogenicity studies in rats and rabbits, observed no effect dose levels were well below those for proposed clinical use (0.3 mg/kg/day for both the rat and rabbit; approximately 0.06X the maximum recommended human 24 hour dose of ribavirin). No maternal toxicity nor effects on offspring were observed in a peri/postnatal toxicity study in rats dosed orally at up to 1 mg/kg/day (estimated human equivalent dose of 0.17 mg/kg based on body surface area adjustment for a 60 kg adult; approximately 0.01X the maximum recommended human 24 hour dose of ribavirin).

Treatment and Post-Treatment Risk to the Fetus: Ribavirin is known to accumulate in intracellular components from where it is cleared very slowly. It is not known whether ribavirin contained in sperm will exert a potential teratogenic effect upon fertilization of the ova. In a study in rats, it was concluded that dominant lethality was not induced by ribavirin at doses up to 200 mg/kg for 5 days (estimated human equivalent doses of 7.14 - 28.6 mg/kg, based on body surface area adjustment for a 60 kg adult; up to 1.7X the maximum recommended human dose of ribavirin). However, because of the potential human teratogenic effects of ribavirin exposure to the fetus, male patients should be advised to take every precaution to avoid risk of pregnancy for their female partners.

It is advised that male patients be counseled to practice effective contraception during treatment with combination INTRON A®/REBETOL® therapy and for the 6 month post therapy period (e.g., 15 half-lives for ribavirin clearance from the body).

Nursing Mothers: It is not known whether INTRON A® (Interferon alfa-2b,

recombinant) and REBETOL® (ribavirin) are excreted in human milk. However, studies in mice have shown that mouse interferons are excreted into the milk. Because of the potential for serious adverse reactions from the drug in nursing infants, a decision should be made whether to discontinue nursing or to discontinue combination INTRON A®/REBETOL® therapy, taking into account the importance of the drug to the mother.

Appendix F: Proposed Phase 4 Post-Marketing Toxicology Studies

Based on discussions between the sponsor (Schering Corp.) and the Division (specifically this reviewer), the following Phase 4 Post-Marketing plan has been agreed to.

The Phase 4 commitment (to generate additional data on the carcinogenic potential of ribavirin) consists of two options, the final selection of which option is to be used being determined after completion of preliminary dose-range finding/feasibility studies by Schering Corp.