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Table 27.Tumor incidences in drug treated vs both controls continued

Table 1: (Continued)

ORGAN/TUMOR TYPE	Males C1	C2	T1	T2	T3	Trend P-value	Females C1	C2	T1	T2	T3	Trend P-value
THYROID GLAND	(50)	(50)	(50)	(50)	(50)		(50)	(50)	(50)	(50)	(50)	
C-Cell Adenoma (B)	5	5	4	3	3	0.862	10	3	5	1	3	0.960
Follicular Cell Adenoma (B)	0	0	0	0	0	NA	0	1	1	0	0	0.862
TESTES	(50)	(50)	(50)	(50)	(49)							
Interstitial Cell Adenoma (B)	0	3	0	1	0	0.894						

LEGEND:
C1 = Control 1
C2 = Control 2
T1 = 150 mg/kg/day SCH 58235 For Male Rats And 50 mg/kg/day SCH 58235 For Female Rats
T2 = 750 mg/kg/day SCH 58235 For Male Rats And 250 mg/kg/day SCH 58235 For Female Rats
T3 = 1500 mg/kg/day SCH 58235 For Male Rats And 500 mg/kg/day SCH 58235 For Female Rats
B = Benign Tumor
NA = No Analysis Performed.

NOTE: For purpose of analysis, the control groups were combined into one group. Also, the parenthetical numbers are the number of animals examined for that organ. The P-values are one-sided and age-adjusted. When P ≤ 0.05, an adjustment for multiplicity is used. No tumor types in Table 1 are statistically significant by the Schering-Plough Research (page 6) or by the Merck & Co., Inc. multi-

Toxicokinetics:
The individual plasma concentrations of SCH 58235 varied greatly at each sampling time-point (mean % coefficient of variation ranged between 8-169%), indicating high inter-animal variability. Plasma concentrations of SCH 58235 poorly correlated with the doses, except at 50-250 mg/kg/day in females, where some increase was noted with the dose. The drug was highly conjugated in plasma and unconjugated represented <1.3% of the total drug. Note that conjugated drug has as much or more drug activity than the active drug itself. The AUC values (0-12 hrs) of only the total drug were provided which did not increase significantly in males 4131, 4434, 4775 ng.h/ml at 150, 750, 1500 mg/kg/day respectively. In females these values were 3326, 6017, 6659 ng.h/ml at 50, 250, 500 mg/kg/day respectively. This may be due to saturation of oral absorption following dietary administration. The values in females were in general similar to those seen in males, even when females received 3-fold lower doses. In a 3-month dietary TK study in rats the AUC values (0-24 hrs) of the total drug were as follows: males 3100, 4700, 7700, 11000 ng.h/ml at 20, 100, 500, 1500 mg/kg/day respectively, these in females at same doses were 1300, 7300, 12000, 13000 ng.h/ml respectively. Overall, increases in plasma concentration were minimal with increases in doses, sponsor states that similar observations were noted in 1-month TK study in rats.

In the current CAC study, $AUC_{0\text{-}12h}$ exposures were provided, however in a metabolism technical summary (volume 10 of the submission, page 93), $\mathbf{AUC_{0\text{-}24h}}$ exposures of the free and total ezetimibe are provided in male and female rats at a high dose of 1500 and 500 mg/kg/day respectively, in males these were 0.130 and 10.12 µg.h/ml respectively. In females these values were 0.130 & 9.47 µg.h/ml respectively.

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Table 28. Toxicokinetics of SCH 58235 in 2-year bioassay in rats

Table 1 Mean Total, Conjugated and Unconjugated SCH 58235 Plasma Concentrations Following Dietary SCH 58235 Administration to Rats (n=4)

Dose (mg/kg) (Male/Female)	Week 4				Week 52			
	Male	%CV	Female	%CV	Male	%CV	Female	%CV
	Total SCH 58235 (ng/mL plasma)							
150/50	389	53	239	36	341[a]	10	349	17
750/250	354	22	692	23	458	40	744	39
1500/500	527	40	464	23	564	41	756	27
	Conjugated SCH 58235 (ng/mL plasma)							
150/50	387	53	236	36	339[a]	9	345	17
750/250	350	23	688	23	456	40	739	39
1500/500	524	40	459	23	562	41	753	28
	Unconjugated SCH 58235 (ng/mL plasma)							
150/50	2.22	57	3.01	41	2.15[a]	87	3.64	37
750/250	3.30	18	4.14	60	1.79	31	4.40	56
1500/500	3.66	33	5.25	9	1.76	92	3.44	54

a: n=3

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:

Again the test model appears to be adequate, because rats are commonly used for carcinogenicity study in other lipid lowering drugs (statins). AUC values (0-12 hrs) of the total drug (parent compound and the glucuronide metabolite) in the current cac study were 4.8 µg.h/ml at 1500 mg/kg/day in males, and 6.7 µg.h/ml in at 500 mg/kg/day in females. In the 3-month dose range study in rats, the AUC exposures (0-24 hrs) were 11 and 12 µg.h/ml at 1500 and 500 mg/kg/day respectively in males and females. Exe cac had approved the doses for rat carcinogenicity study based on saturation of absorption of the drug which was achieved in the current study in male rats at the lowest dose of 150 mg/kg/day, as exposures did not further increase with increase in dose to 750-1500 mg/kg/day (4.1, 4.4, 4.8 µg.h/ml at 150, 750, 1500 mg/kg/day respectively). While in females, saturation of absorption was observed at a mid dose of 250 mg/kg/day (values were 3.3, 6.0, 6.7 µg.h/ml at 50, 250, 500 mg/kg/day respectively). Only toxicity noted was in males, where the drug produced decrease in weight gains at mid-high doses. In summary, originally proposed high doses (of 1500 mg/kg/day in males and 500 mg/kg/day in females) appeared to be reasonable, however these did not produce significant toxicity/mortality or carcinogenicity in rats.

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Evaluation of tumor findings:

In females, hepatocellular adenomas were observed in 2/50 animals (or 4%) at a high dose vs none in control or other groups. Sponsor states that these are in the

range of historical control values (range 1-5.5%), however the reference for historical control data appears to be in the rats which are not diet restricted. In conclusion oral dosing for 2 years at 150, 750, 1500 mg/kg/day in males and 50, 250, and 500 mg/kg/day in females did not result in significant increase in neoplastic or non-neoplastic findings. However, this is contingent on follow up of the two hepatocellular adenomas in the high dose female group, which need to be compared with the historical controls in diet restricted rats, and the data for these have not been provided, and are requested from the sponsor by statistician reviewer. In the large intestine, malignant lymphoma (1/50 female rats at mid dose vs none in control or other groups) and fatal fibrosarcoma, mesentery (in 1/50 males at mid dose vs none in control or other groups) were observed, but these were again not statistically significant. A decrease in BW gains of 7-10%. was observed in males at mid/high doses during most of the study weeks. In a 3-month dose range study in non–diet restricted rats (at doses up to doses of 1500 mg/kg/day) or a 2-week TK study in diet restricted rats (at 2000 mg/kg/day), no effect on BW or weight gains was observed. Similarly a 6-month dietary toxicity study in rats did not show effects on BW or weight gains. However, a decrease in BW (4-7%) and weight gains (of 7-10%) was observed in the present cac study in males at 750-1500 mg/kg/day during most of the study weeks. Therefore, a no-effect dose in 2-year rat study in males was 150 mg/kg/day and in females was 500 mg/kg/day.

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Executive CAC Meeting Minutes finalized and signed off on 8/2/02
Date of Meeting: April 16, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abigail Jacobs, Ph.D., HFD-540, Alternate Member
Robin Huff, Ph.D., HFD-570, Alternate Member
Karen Davis Bruno, Ph.D., HFD-510, Team Leader
Indra Antonipillai, Ph.D., HFD-510, Presenting Reviewer

Author of Draft: Indra Antonipillai

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA #: NDA 21-445, IND [redacted]
Drug Name: Zeita (Ezetimibe) tablets
Sponsor: MPS Singapore CO., LLC, Singapore. Joint venture between Merck & Co. and Schering Corp.

Background:
Zeita is a cholesterol absorption inhibitor. Its mechanism of action is unknown but it acts locally in the intestine to block the intestinal absorption of cholesterol and related phytosterols. It is indicated alone, or in combination with statins for primary hypercholesterolemia (heterozygous [redacted] familial) and for homozygous familial sitosterolemia. The drug is mainly metabolized to glucuronide, which has as much or more drug activity than the drug itself.

Mouse carcinogenicity study

In a 2-year carcinogenicity study in mice (Crl:CD(CR)BR, 50/sex/dose), doses of 25, 100, 500 mg/kg/day were administered in a diet for 104 weeks. No AUC values of the total drug (parent + glucuronide metabolite) were provided but plasma concentrations of the total drug were provided which increased with doses (males 0.21, 1.8, and 14.1 µg/ml, females 0.61, 5.7 and 31.3 µg/ml at 25, 100, 500 mg/kg/day respectively).

The Exe Cac Committee had previously concurred with the sponsor on doses selected for the present cac study. The highest dose selection (500 mg/kg/day) for mouse CAC study was based on saturation of exposure to the parent drug, which at 500 and 2000 mg/kg/day was 0.98 and 0.95 µg.h/ml in males, and 0.33 and 0.35 µg.h/ml in females respectively in a 3-month dose range study in mice. At 500 mg/kg/day, the exposure to parent compound and the major metabolite glucuronide (males 216, females 347 µg.h/ml) was 166-267 fold the human dose at 20 mg/day (1.3 µg.h/ml, based also on AUC of a parent + metabolite). The current clinical dose in humans is 10 mg/day. Also the dietary route was chosen, as exposures of the parent drug + metabolite were higher by this route [redacted] µg.h/ml) vs. gavage [redacted] µg.h/ml).

In the present carcinogenicity study, no AUC values of the total drug (parent + glucuronide) were provided to determine if the saturation of absorption was achieved, as was concurred by the Exe. CAC committee in the dose selection protocol. However in

the present study approximately 3 fold higher plasma concentrations were achieved (males 14.1 µg.h/ml, females 31.3 µg.h/ml) vs in the 3-month mouse study (males 4.4, females 9.2 µg/ml) which already showed exposures 166-267 fold the human doses.

No neoplastic findings were observed in mice with the drug compared to control. Oral dosing for 2 years at 25, 100 and 500 mg/kg/day did not result in a significant increase in neoplastic or non-neoplastic findings in mice. In females, malignant histiocytic sarcoma (at undetermined primary site) was observed at a higher number in 25 mg/kg/day group (4, 7, 11, 3 and 6 at 0, 0, 25, 100, 500 mg/kg/day respectively), but no dose related trend was observed, and was not statistically significant. In males in harderian gland, increased incidences of benign adenomas were noted specially at low doses (0, 2, 9, 3, 4 at 0, 0, 25, 100, 500 mg/kg/day respectively, in females these incidences were 2, 3, 3, 1, 4 respectively), but were not statistically significant in the trend analysis.

Rat carcinogenicity study

A 2-year dietary carcinogenicity study in rats (Crl:CD (SD)BR, 50/sex/dose) was conducted, where doses of 150, 750, 1500 mg/kg/day were administered to males, and 50, 250, 500 mg/kg/day to females in a diet for 104-106 weeks. All animals were diet restricted and received 25% less food/day. AUC values (0-12 hrs) of the total drug (parent + glucuronide metabolite) poorly correlated with doses (males 4.1, 4.4, 4.8 µg.h/ml at 150, 750, 1500 mg/kg/day respectively, females 3.3, 6.0, 6.7 µg.h /ml at 50, 250, 500 mg/kg/day respectively).

Again, the Exe Cac Committee had concurred with the sponsor's dose selection for the present cac study. The highest dose selection in the rat CAC study (1500 mg/kg/day in males and 500 mg/kg/day in females) was based on saturation of exposure ($AUC_{0\text{-}24h}$) to the parent drug + glucuronide in a 3-month study in rats, which was achieved at 1500 mg/kg/day in males (AUC exposures were 3.1, 4.7, 7.7, 11 µg.h/ml at 0, 20, 100, 500, 1500 mg/kg/day) and at 500 mg/kg/day in females (1.3, 7.3, 12, 13 µg.h/ml respectively), as no increases in plasma conc. occurred after 500 mg/kg/day. However these values provided the exposures of only 6-9 fold the human doses at 20 mg/day (1.3 µg.h/ml, based also on AUC of a parent + metabolite). In a 2-week study in diet restricted rats (with 25% less food), exposure to the total drug was not different at 2000 mg/kg/day (males 10.6, females 15.8 µg.h/ml) compared to that in a 3-month study in non-diet restricted rats at 1500 mg/kg/day (males 11, females 13 µg.h/ml), suggesting that plateau in exposure had been reached in males and females. Also the dietary route was chosen, as exposures of the parent drug + metabolite were higher by this route (——— µg.h/ml) vs. gavage (— µg.h/ml).

In the current rat carcinogenicity study, saturation of absorption of the drug ($AUC_{0\text{-}12h}$) was achieved in the male rats at the lowest dose of 150 mg/kg/day, as exposures did not further increase with increase in dose to 750-1500 mg/kg/day (4.1,4.4, 4.8 µg.h/ml at 150, 750, 1500 mg/kg/day respectively), while in females this was achieved at a mid dose of 250 mg/kg/day (values were 3.3, 6.0, 6.7 µg.h/ml at 50, 250, 500 mg/kg/day respectively).
The drug decreased body weights (of 4-7%) and weight gains (of 7-10%) in males at mid/high doses during most of the study weeks. In female rats, hepatocellular adenomas were observed in 2/50 animals (or 4%) at high dose vs. none in control or other groups. Sponsor states (in the initial submission on 12/27/2001) that these are in the range of

historical control values (range 1-5.5%). However the reference for historical control data appears to be in the rats which are not diet restricted (Spontaneous neoplastic lesions and selected non-neoplastic lesions in the Crl:CDBR rats, [redacted]. Sponsor states that no statistically significant trend in the incidence of tumor-bearing rats was observed with increases in drug doses, and the drug (SCH 58235) tested negative in both sexes (up to doses of 1500 mg/kg/day in males, and 500 mg/kg/day in females) in the 2-year rat carcinogenicity study.

In summary, oral dosing for 2 years in rats at 150, 750, 1500 mg/kg/day in males and 50, 250, and 500 mg/kg/day in females did not result in significant increase in neoplastic or non-neoplastic findings. However, this is contingent on follow up of the two hepatocellular adenomas observed in the high dose female group, which was communicated to the sponsor in a T-con on 5/23/2002.

In a subsequent 7/3/2002 submission, sponsor has provided the historical tumor incidences data in control diet restricted rats. No hepatocellular adenomas were observed in male or female animals in control diet restricted rats. The sponsor considers the [redacted] databases more appropriate historical control data set for ezetimibe studies since their own database is limited to 100 diet restricted rats/sex/group. The [redacted] databases in the diet restricted rats (Spontaneous neoplastic lesions and survival in the Crl:CD(SD) BR rats maintained on dietary restriction, [redacted] show that incidence of hepatocellular adenomas in female rats are in the range of 0-8% (mean 2.2%) which is derived from 26 total studies, and in 20 studies lesions were identified. This suggests that hepatocellular adenomas of 4% in the current carcinogenicity study in female rats are incidental.

Executive CAC Recommendations and Conclusions:

Mouse

The study protocol was acceptable, as it had received prior concurrence from the Exec CAC committee. The Committee concluded that there were no significant tumor findings in the 2-year mouse CAC study.

Rat:

The study protocol was acceptable, having received prior concurrence from the Exec CAC committee. After reviewing both the sponsor's and [redacted] historical control dataset in diet restricted rats, the committee concluded that a 4% increase in tumor incidences in hepatocellular adenomas in female rats at a high dose was incidental. In conclusion, The Committee concurred that there were no significant tumor findings in a 2-year diet restricted rat CAC study.

Joseph Contrera, Ph.D., 8/2/02
Acting Chair, Executive CAC

cc:/

/Division File, HFD-510, NDA 21-445
/Team leader, HFD-510 Davis Bruno
/Reviewer, HFD-510 Antonipillai
/CSO, HFD-510, Koch
/HFD-024, ASeifried

VII. A. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY WITH MONOTHERAPY:

1. Segment I study: Effects of oral SCH 58235 (by gavage) on fertility study in Rats

Key study findings: : In the rat fertility study (0, 250, 500, 1000 mg/kg/day), the drug at the highest dose of 1000 mg increased clinical signs in females (chromorhinorrhea, reduced fecal pellet or no stool, urogenital staining) prior to gestation and during gestation. SCH 58235 increased resorptions (7.5% vs 4% in controls) in females, but these effects were not significant and/or within the historical control incidences . However, decreased fertility index was observed in both sexes (84% vs 96% in controls). Doses of 250-500 mg/kg/day produced some broken or loose teeth in both sexes, but did not have any affect on the fertility or on the general maternal or paternal reproductive performance, or on the progression of pregnancy in rats. **NOAEL doses of SCH 58235 in this study were 500 mg/kg/day** (or 500 times the human dose of 10 mg/day, based on body surface area

Study no.: 96381
Volume #, and page #: 1.36, pg.1 (reference 23)
Conducting laboratory and location: Safety Evaluation Center/Schering-Plough Research Center; Layfayette, NJ
Date of study initiation: 6/19/1997
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SZ-58235-97-X-101

Formulation/vehicle: 0.4% aq. methylcellulose

Methods:

Species/strain: Rat (Crl:CD(SD)VAF/PLUS
Doses employed: 0, 250, 500, 1000 mg/kg SCH 58235
Route of administration: oral gavage
Study design: 0, 250, 500, 1000 mg/kg SCH 58235 were given to male rats from 21 days prior to, and throughout mating, and to female rats from 14 days prior to mating, until day 7 of pregnancy. Males were sacrificed approximately 3 weeks after the beginning of the mating period and females on day 14 after mating, and necropsied.

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Study Design:

Oral Study of Fertility of SCH 58235 in Rats (P-6846): **Study Design**					
Group	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	Number of Rats	
				Males	Females
Vehicle Control (0.4% methylcellulose)	0	5	0	25	25
Low-Dose (SCH 58235)	250	5	50	25	25
Mid-Dose (SCH 58235)	500	5	100	25	25
High-Dose (SCH 58235)	1000	5	200	25	25

Oral Study of Fertility and Early Embryonic Development of SCH 58235 in Rats (P-6846) **Observations and Measurements**	
Investigation	Performed
Estrous Cycle - Females	Daily for one week prior to mating until evidence of mating
Clinical Observations	Daily
Food Consumption - Males Food Consumption - Females	Weekly during the premating period Weekly prior to mating and over gestation Days 0-7 and 7-14
Body Weights - Males Body Weights - Females	Twice weekly Twice weekly prior to mating and on Days 0 (day of mating) 4, 7 and 14 after mating
Drug Consumption (calculated)	Weekly
Necropsy – Males Necropsy/C-Section – Females	After completion of the mating period Gestation Day 14

Results:

Mortality: At 500 mg/kg/day one female was found dead after 7 days, the death was considered accidental

Clinical signs: In males: chromodacryorrhea was observed in two males (at low/mid doses), no stool in one male (at mid dose), and scabs in 4 males (3 at low dose + 1 at mid dose). Broken/loose teeth in three rats (one each at 0, 250, 500 mg/kg/day)

Females: During premating, chromorhinorrhea was observed in 3 females (1 at mid + 2 at a high dose), some blood in litter pan in 1 animal and reduced fecal pellets (at a high dose)

Females during gestation, chromorhinorrhea was observed in 4 females (2 at low + 2 at a high dose), some blood in litter pan in 1 animal and reduced fecal pellets (at a high dose), reduced fecal pellets and urogenital staining (in 1 animal at a high dose). Broken teeth in two (one each at 250 & 500 mg/kg/day)

Body weight/weight gains: No drug related effects were observed

Food consumption: No drug related effects on food consumption were observed.

Estrous cycle determination: these were similar in treated vs controls.

Toxicokinetics: Not examined

Terminal and necroscopic evaluations:

Dams: **Less number of animals were pregnant at a high dose** (24/25, 22/25, 22/25, 21/25 at 0, 250, 500, 1000 mg/kg/day). None of the above females had any clinical signs, except one animal at 1000 mg/kg/day had chromorhinorrhea, blood in litter pan, and reduced number of fecal pellets. Abscesses in the left axillary area were noted in two females (1 at 500 + 1 at 1000 mg/kg/day) at necropsy.

Mating and fertility indices: Both male fertility index (96, 88, 92, 84% respectively) and female fertility index (96, 88, 92, 84% respectively) was decreased at a high dose.

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Table 11 Mating and Fertility Indices

Parameter	Dose Groups (mg/kg)			
	0 (SCH 58235)	250 (SCH 58235)	500 (SCH 58235)	1000 (SCH 58235)
[a] Male Mating Index	96% (24/25)	100% (25/25)	100% (25/25)	100% (25/25)
[b] Female Mating Index	100% (25/25)	100% (25/25)	100% (24/24)	100% (25/25)
[c] Male Fertility Index	96% (23/24)	88% (22/25)	92% (22/24)	84% (21/25)
[d] Female Fertility Index	96% (24/25)	88% (22/25)	92% (22/24)	84% (21/25)

a: Male Mating Index (%) =
Number of males with evidence of mating* X 100
Total number of males used for mating

b: Female Mating Index (%) =
Number of females with evidence of mating X 100
Total number of females used for mating

c: Male Fertility Index (%) =
Number of males siring at least one litter X 100
Number of males with evidence of mating

d: Female Fertility Index (%) =
Number of Females with confirmed pregnancy
Total number of females with evidence of mating

* Sperm in vaginal smear and/or vaginal plug

Reproduction parameters: The mean total resorptions were increased at a high dose, the number/animal was 0.6, 0.8, 1.0, 1.2 at 0, 250, 500, 1000 mg/kg/day (or 4, 5.4, 6.4, 7.5% respectively). The sponsor states that it was within the historical range of their laboratory (3.8-11.2 %). The mean number of corpora lutea were increased at a mid dose (15.1, 15.4, 16.8*, 16.4, *p<0.05), this the sponsor explains is not likely due to the drug because it is not dose related and the control mean in this study is below the historical mean in their laboratory (16.2%, Jan 1984-July 1998). The preimplantation loss was higher at a high dose (2.5, 2.1, 3.5, 4% respectively)

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Table . Reproduction data in segment I study in female rats

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ORAL STUDY OF FERTILITY OF SCH 58235 IN RATS
SUMMARY OF REPRODUCTION DATA

	DOSAGE		0 MG/KG	250 MG/KG	500 MG/KG	1000 MG/KG
Pregnant used for calculation		N	24	22	22	22
Corpora Lutea		N	343	339	370	367
No. per animal		MEAN	15.1	15.9	16.8*	16.5
		S.D.	3.17	1.87	2.00	1.68
Preimplantation Loss		N	9	7	13	10
% per group		%	2.5	2.1	3.5	2.8
% per animal		MEAN	2.6	3.1	3.1	3.7
		S.D.	6.15	4.66	6.27	12.22
Implantation Sites		N	334	332	357	333
No. per animal		MEAN	14.0	15.1	16.2	15.9
		S.D.	3.23	2.02	1.72	2.03
Fetuses		N	340	314	334	308
No. per animal		MEAN	14.2	14.3	15.2	14.7
		S.D.	3.17	1.93	2.56	2.01
Alive		%	100.0	100.0	100.0	100.0
Dead		%	0.0	0.0	0.0	0.0
Live Fetuses		N	340	314	334	308
No. per animal		MEAN	14.2	14.3	15.2	14.7
		S.D.	3.17	1.93	2.56	2.01
Dead Fetuses		N	0	0	0	0
No. per animal		MEAN	0.0	0.0	0.0	0.0
		S.D.	0.00	0.00	0.00	0.00
% of impl. per group		%	0.0	0.0	0.0	0.0
% of impl. per animal		MEAN	0.0	0.0	0.0	0.0
		S.D.	0.00	0.00	0.00	0.00

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

Organ weights were not different between treated vs controls.

Summary of Segment I rat study with SCH 58235: In a segment I fertility study in rats, animals were given oral (by gavage) SCH 58235 at doses of 0, 250, 500, 1000 mg/kg/day. Females were given the drug for 2-weeks prior to mating, throughout mating, and from days 0 to 7 of gestation, and sacrificed on GD-14. Males were given the drug for 3 weeks prior to mating, during mating, until necropsy. Clinical signs were observed in both sexes before mating (chromodacryorrhea, no stool, scabs in 1-4 rats at low/mid doses in males, and in females at mid/high doses) during gestation in females (the above signs were observed in 2 and 4 females at mid/high doses vs in 1 and 3 females during premating period). Less animals were pregnant at higher doses (male/female fertility index was 96, 88, 92, 84% respectively, but these values were not significantly different. The percentage of resorptions were higher at a high dose (4, 5.4, 6.4, 7.5% respectively), but within the sponsor's historical control range. In males, mean weights of testes and epididymides in drug treated were comparable to control, these tissues or prostate were not examined for histopathological exams. No other drug related effects on general maternal or paternal reproductive performance, or on the progression of pregnancy in rats were observed. The NOAEL doses in this fertility study in male and female rats were 1000 mg/kg/day (or 500 times the human dose of 10 mg/day, based on body surface area). Sponsor's NOAEL is 1000 mg/kg/day. Maternal exposures were not examined in this study.

2. Segment II study: Effects of oral SCH 58235 (by gavage) on embryo-Fetal Developmental in Rats

Key study findings:
Maternal NOAEL was 1000 mg/kg/day, based on absence of toxicity even at the highest dose. **Embryo-fetal NOAEL was 250 mg/kg/day**, based on increased skeletal variations at a high dose and malformation in the tail at a mid dose (malformations in the tail have also been observed in the segment II study in rabbits and segment III study in rats). Malformations in the heart, tail and kidney were observed at low-mid doses in 1-2 fetuses. Malformations included a small size heart (with shortened mandible and maxilla in one fetus and litter at 250 mg/kg/day), short filamentous tail (in one fetus and litter at 500 mg/kg/day), vestigial right kidney (in one fetus and litter at 500 mg/kg/day). Soft tissue variations were observed in one fetus at low dose (anasarca or generalized massive edema), and in 3 fetuses (a dilated renal pelvis in two at a low dose and 1 at a high dose). Increased skeletal observations were noted at a high dose. These included extra pair of thoracic ribs (fetal incidences 11 vs 7 in controls), unossified cervical vertebral centra (fetal incidences 82 vs 38 in controls, litter incidences 23 vs 14 in controls), and shortened ribs (fetal incidences 4 vs 2 in controls, litter incidences 2 vs 1 in controls). The skeletal findings may be dose related as these were above historical control fetal means, but visceral malformations/variations may not be dose related.

Study no.: 96383
Volume #, and page #: 1.36, pg.1 (reference 25)
Conducting laboratory and location: Safety Evaluation Center/Schering-Plough Research Center; Layfayette, NJ
Date of study initiation: 10/4/1996
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 95-58235-ZZX-05

Formulation/vehicle: 0.4% aq. methylcellulose

Methods:

Species/strain: Rat (Crl:CD(SD)VAF/PLUS
Doses employed: 0, 250, 500, 1000 mg/kg SCH 58235
Route of administration: oral gavage
Study design: 0, 250, 500, 1000 mg/kg/day of SCH 58235 were given from day 6 through day 15 of pregnancy (n=25/dose). Animals were necropsied on day 21.

Study Design:

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Oral Embryo-Fetal Development Study of SCH 58235 In Rats (P-6515): Study Design				
	SCH 58235 (Batch No. 95-58235-ZZX-05)			
Group	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	No. of Females[a]
Control (0.4% methylcellulose)	0	5	0	25
Low-Dose (SCH 58235)	250	5	50	25
Mid-Dose (SCH 58235)	500	5	100	25
High-Dose (SCH 58235)	1000	5	200	25
a: All female rats were dosed daily on Days 6-15 after mating.				

Oral Embryo-Fetal Development Study of SCH 58235 In Rats (P-6515): Observations and Measurements			
Investigation	Performed	Investigation	Performed
Clinical Observations	Daily	Reproduction Parameters	Yes
Body Weights	Gestation Days 0, 6, 9, 12, 15, 18, 21	Fetal Body Weights	Day 21
Food Consumption	Gestation Days 0-6, 6-10, 10-15 and 15-21	Fetal Gross/Soft Tissue/ Skeletal Examinations	Yes
Necropsy/C-Section	Gestation Day 21		

Toxicokinetics: A separate study was conducted to assess the systemic exposure of the drug in Segment II study in rats (vol 1.183, page 1, reference 15, study # 99373). The drug was given by gavage on gestation day 6 through day 15 (n=8/dose). Drug batch Number was 97-58235-X-02.

Study design for segment II TK study in rats

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Toxicokinetic Study of Orally Administered SCH 58235 In Pregnant Rats (SN 99373): **Study Design**				
Group	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	Number of Females
Low-Dose (SCH 58235)	250	5	50	8
Mid-Dose (SCH 58235)	500	5	100	8
High-Dose (SCH 58235)	1000	5	200	8

Toxicokinetic Study of Orally Administered SCH 58235 In Pregnant Rats (SN 99373): **Observations and Measurements**	
Investigation	Performed
Viability Check	Daily
Clinical Observations	Daily
Body Weight	Gestation Days 0, 6, 9, 12, and 15
Plasma Analysis for SCH 58235	Gestation Day 15: Either 1, 4, and 12 hours postdose, or at 2, 8 and 24 hours postdose
Pregnancy Status	At necropsy on gestation Day 15 or 16

Results:

Mortality: None

Clinical signs: No drug related effects were observed
Body weight/weight gains: unremarkable in all groups

Food consumption: No drug related effects on food consumption were observed.

Toxicokinetics: As indicated a separate study as mentioned above. The plasma AUC values are shown in the Table. There was extensive glucuronidation of the drug in rats. Exposure to the unconjugated or free drug was less than 2% of the total exposure. The AUC values did not proportionally increase with the doses. The AUC of total drug (parent + metabolite) at 250, 500, 1000 mg/kg/day were 3.1, 4.2, 4.9 μg.h/ml respectively on day 15 of gestation in rats

Tk data on gestation day 15 in segment II study in rats

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Toxicokinetic Parameters for Total, Conjugated, and Unconjugated SCH 58235 on Day 15 after Oral (Gavage) Administration of SCH 58235 in Pregnant Rats			
	Toxicokinetic Parameters		
Dose (mg/kg)	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) (ng•hr/mL)
	Total SCH 58235		
250	521	2	3091
500	489	4	4228
1000	629	1	4929
	Conjugated (Total minus Unconjugated) SCH 58235		
250	518	2	3099
500	485	4	4312
1000	1047	1	5302
	Unconjugated SCH 58235		
250	2.69	4	26.9
500	4.11	4	NR[a]
1000	5.67	4	47.8
a: NR = Not reportable; Percent AUC extrapolated is greater than 25			

Terminal and necroscopic evaluations: No drug related necropsy observations were seen

Dams: No significant effects on pregnancy rates (24/25, 22/25, 24/25, 25/25 were pregnant), sex distribution, distribution in uterus, effects on corpora lutea, or on implantations were observed. The mean resorptions were not significantly effected (0.54, 0.45, 0.46, 0.28 at 0, 250, 500, 1000 mg/kg/day, or 3.5%, 2.9%, 2.8%, 1.8% respectively).

Fetal Gross observations: Following malformations were noted in fetuses:
Small heart (nonfunctional) **and kidney** (1/4th the normal size, bilateral, nonfunctional) in one fetus and litter at 250 mg/kg/day.
Anasarca in one fetus and litter at 250 mg/kg/day
Short filamentous tail (this fetus had no caudal vertevrae) in one fetus and litter at 500 mg/kg/day.
Kidney (vestigial right kidney, it was found anterior to bladder, the left kidney was horse shoe shaped) at 500 mg/kg/day in1 fetus, 1 litter

Fetal skeletal observations. Extra pair of thoracic ribs (fetal incidences 7, 5, 3, 11, litter incidences 6, 4, 2, 6 respectively), unossified cervical vertebral centra (fetal incidences 38, 71, 43, 82, litter incidences 14, 21, 13, 23 respectively), and shortened ribs (fetal incidences 2, 0, 2, 4, litter incidences 1, 0, 2, 2 respectively) were generally all increased at a high dose. Total skeletal variations of fetal incidences were not significant in treated vs controls (68.3, 72, 62, 76% respectively). Reduced ossification of the sternebrae were not significantly different in treated vs controls. However, none of the above skeletal findings are considered significant by the sponsor, as they claim there is considerable variation in rats and are found in all groups

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Table: Summary of fetal gross observations:

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ORAL EMBRYO-FETAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
SUMMARY OF FETAL GROSS OBSERVATIONS : TOTAL (PERCENT)
SCHEDULED C-SECTION

DOSE GROUP	0 MG/KG	250 MG/KG	500 MG/KG	1000 MG/KG
LITTERS EVALUATED	24	22	24	25
FETUSES EVALUATED	358	336	382	388
LIVE	357	336	382	388
DEAD	1	0	0	0
M SMALL HEART				
FETAL INCIDENCE	0	1(0.3)	0	0
LITTER INCIDENCE	0	1(4.5)	0	0
M SMALL KIDNEY				
FETAL INCIDENCE	0	1(0.3)	0	0
LITTER INCIDENCE	0	1(4.5)	0	0
V ANASARCA				
FETAL INCIDENCE	0	1(0.3)	0	0
LITTER INCIDENCE	0	1(4.5)	0	0
M SHORT FILAMENTOUS TAIL				
FETAL INCIDENCE	0	0	1(0.3)	0
LITTER INCIDENCE	0	0	1(4.2)	0
TOTAL FETAL GROSS OBSERVATIONS *				
FETAL INCIDENCE	0	1(0.3)	1(0.3)	0
LITTER INCIDENCE	0	1(4.5)	1(4.2)	0

M-MALFORMATION V-VARIATION
* THE TOTAL REPRESENTS THE TOTAL NUMBER OF OFFSPRING WITH OBSERVATIONS.
THIS NUMBER MAY BE LESS THAN THE SUM OF ALL FINDINGS AS SOME OFFSPRING MAY HAVE MULTIPLE FINDINGS

Variations: Soft tissue variations were observed in one fetus at low dose (anasarca), and a dilated renal pelvis in 3 fetuses (two at a low dose and one at a high dose).

ORAL EMBRYO-FETAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
SUMMARY OF SOFT TISSUE OBSERVATIONS : TOTAL (PERCENT)
SCHEDULED C-SECTION

DOSE GROUP	0 MG/KG	250 MG/KG	500 MG/KG	1000 MG/KG
LITTERS EVALUATED	24	22	24	25
FETUSES EVALUATED	172	161	184	188
LIVE	172	161	184	188
DEAD	0	0	0	0
M VERTICIAL KIDNEY				
FETAL INCIDENCE	0	0	1(0.5)	0
LITTER INCIDENCE	0	0	1(4.2)	0
M HORSESHOE KIDNEY				
FETAL INCIDENCE	0	0	1(0.5)	0
LITTER INCIDENCE	0	0	1(4.2)	0
V DILATED RENAL PELVIS (papilla rated 0 or 4)				
FETAL INCIDENCE	0	3(1.9)	0	1(0.5)
LITTER INCIDENCE	0	2(9.1)	0	1(4.0)
TOTAL SOFT TISSUE OBSERVATIONS *				
FETAL INCIDENCE	0	3(1.9)	1(0.5)	1(0.5)
LITTER INCIDENCE	0	2(9.1)	1(4.2)	1(4.0)

M-MALFORMATION V-VARIATION
* THE TOTAL REPRESENTS THE TOTAL NUMBER OF OFFSPRING WITH OBSERVATIONS.
THIS NUMBER MAY BE LESS THAN THE SUM OF ALL FINDINGS AS SOME OFFSPRING MAY HAVE MULTIPLE FINDINGS

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Summary of Segment II rat study with SCH 58235: In a segment II teratology study in rats, pregnant animals (n=25/group) were given oral SCH 58235 by gavage at doses of 0, 250, 500, 1000 mg/k/day from day 6 to day 15 of gestation. Females were sacrificed

on day 21 PC and necropsied. In females, no maternal toxicity was observed even at the highest dose. At 250-500 mg/kg/day some malformations were observed in fetuses and litters. These included a small size heart (with shortened mandible and maxilla in one fetus and one litter at 250 mg/kg/day), short filamentous tail (in one fetus and one litter at 500 mg/kg/day), and vestigial right kidney (in one fetus and one litter at 500 mg/kg/day. No malformations were observed in control or high dose rats. The fetal findings (visceralvariations/malformations) although of lower incidences and not dose related, may not be incidental. Soft tissue variations were observed in one fetus at low dose (anasarca), and in 3 fetuses (a dilated renal pelvis), 2/3 at a low dose and 1/3 at a high dose. Increased skeletal observations were noted at a high dose. These included extra pair of thoracic ribs (fetal incidences 11 vs 7 in controls, or 5.5% vs 3.8% in controls, 1.9% historical fetal mean), unossified cervical vertebral centra (fetal incidences 41% vs 20% in controls, litter incidences 92% vs 56% in controls, 49% historical fetal mean), and shortened ribs (fetal/litter incidences 2% vs 1% in controls, o.3% historical fetal mean). Sponsor states that malformations are not drug related as they varied and showed no dose related increase, similarly soft tissue variations are not unusual in this species, and skeletal variations are scattered so they are not drug related as well, however the skeletal effects may be drug related. The AUC of the parent + metabolite at 1000 mg/kg/day in pregnant rats on gestation day 15 (4.9 µg.h/ml) was 8 - fold, the human AUC at 10 mg/day (0.68 µg.h/ml).

Note that the human AUC values are calculated from two 14-days 10 mg/day multiple dose studies, with $AUC_{0\text{-}24h}$ values of 677 and 681 ng.h/ml respectively, mean were 682 ng.h/ml (or 0.68 µg.h/ml). The human AUC values at 20 mg/day is 1.314 µg.h/ml.

Maternal NOAEL was 1000 mg/kg/day in segment II study in rats, as no toxicity was observed even at the highest dose of 1000 mg/kg/day. **The embryo-fetal NOAEL was 250 mg/kg/day** (≈ 250 times the human dose of 10 mg/day, based on body surface area), since increased skeletal variations were noted at the highest dose of 1000 mg/kg/day and malformation in the tail (short filamentous tail) was observed at a mid dose. Malformations in the tail have also been observed in the segment II study in rabbits and segment III study in rats. Some developmental toxicity (increased malformations in head, kidney or soft tissue variations) was noted in 1-2 fetuses at 250-1000 mg/kg/day, but there was no dose related trend. Fetal exposures were not examined in this study, but the AUC of the parent + metabolite at 250 mg/kg/day in rats on gestation day 15 (3.1 µg.h/ml) was 4-fold, the human AUC at 10 mg/day (0.68 µg.h/ml).

In summary, developmental NOAEL was 250 mg/kg/day (or 4-fold the human AUC at 10 mg/day, or ≈ 250-fold the human dose of 10 mg/day based on body surface area) and maternal NOAEL was 1000 mg/kg/day (or 8-fold the human AUC at 10 mg/day, or ≈ 1000-fold the human dose of 10 mg/day based on body surface area). Sponsor's NOAEL's are 1000 mg/kg/day in segment II study in rats for both maternal and embryo fetal toxicity

Segment II study: Effects of oral SCH 58235 (by gavage) on embryo-Fetal Developmental in Rabbits

Key study findings:
Maternal NOAEL was 500 mg/kg/day, based on increased resorptions in females (9.9% vs 4.1% in controls) and altered sex distribution (ratios of male/female fetuses was increased at MD & HD 1.2 & 1.4 vs 0.96 in controls). **Embryo-fetal NOAEL was <250 mg/kg/day**, based on increased malformations in the head, tail and visceral area and increased skeletal variations seen at all doses. Malformations included, exencephaly-agenesis of tail (in one fetus and litter, both at 250 mg/kg/day, head malformed (in one fetus and litter at 500 mg/kg/day), omphalocele (in 1, 3, 1, 2 fetuses and litters respectively), and shortened tail (in one fetus and litter) at 1000 mg/kg/day. In addition skeletal variations were observed, these included extra pair of thoracic ribs which were increased at all doses (fetal incidences 67, 107, 121, 90, litter incidences 14, 19, 18, 16 respectively), focal thickening of ribs at mid/high doses (fetal/litter incidences 0, 0, 2, 1), unossified distal humeral epiphysis were higher at a mid dose (fetal 1, 0, 4, 0, litter 1, 0, 2, 0). Scoliosis was observed in 1 fetus & litter at a high dose (fetal incidences 0.8, litter incidences 5.9 vs none in other groups). However, sponsor states that all these are not different from controls and NOAEL for maternal and fetal toxicity is 1000 mg/kg/day. Maternal NOAEL was 500 mg/kg/day (or 140-fold the human dose of 10 mg/day based on exposures, and ≈ 1000-fold the human dose of 10 mg/day based on body surface area). The embryo-fetal NOAEL was 250 mg/kg/day (or 100-fold the human dose of 10 mg/day based on exposures, and ≈ 500-fold the human dose of 10 mg/day based on body surface area).

Study no.: 96385
Volume #, and page #: 1.42, pg.1 (reference 29)
Conducting laboratory and location: Safety Evaluation Center/Schering-Plough Research Center; Layfayette, NJ
Date of study initiation: 10/4/1996
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 95-58235-ZZX-05

Formulation/vehicle: 0.4% aq. methylcellulose

Methods:

Species/strain: NZW
Doses employed: 0, 250, 500, 1000 mg/kg SCH 58235
Route of administration: oral gavage
Study design: 0, 250, 500, 1000 mg/kg SCH 58235 were given from day 7 through day 19 of pregnancy. Animals necropsied on day 30.

Study Design:

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Oral Embryo-Fetal Development Study of SCH 58235 in Rabbits (P-6516): **Study Design**				
	SCH 58235 (Batch No. 95-58235 ZZX-05)			
Group	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	Number of Females[a]
Control (0.4% aqueous methylcellulose)	0	5	0	20
Low-Dose (SCH 58235)	250	5	50	20
Mid-Dose (SCH 58235)	500	5	100	20
High-Dose (SCH 58235)	1000	5	200	20
a: All female rabbits were dosed daily on Days 7-19 after mating.				

Parameters evaluated

Oral Embryo-Fetal Development Study of SCH 58235 in Rabbits (P-6516): **Observations and Measurements**			
Investigation	Performed	Investigation	Performed
Clinical Observations	Daily	Reproduction Parameters	Yes
Body Weights	Days 0, 7, 10, 13, 16, 19, 22, 25, 28, 30	Fetal Body Weights	Day 30
Food Consumption	Days 0-30	Fetal Gross/Soft Tissue/Skeletal Examinations	Yes
Necropsy/C-Section	Day 30		

Toxicokinetics: A separate study was conducted to assess the systemic exposure of the drug in Segment II study in rabbits (vol 1.185, page 1, reference 17, study # 99291). The drug was given by gavage on gestation day 7 through day 19 (n=8/dose). Drug batch Number was 97-58235-X-02.

Study design for segment II TK study in rabbits

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Toxicokinetic Study of Orally Administered SCH 58235 In Pregnant Rabbits (SN 99291): **Study Design**				
Group	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	Number of Females
I (SCH 58235)	250	5	50	4
II (SCH 58235)	500	5	100	4
III (SCH 58235)	1000	5	200	4

Toxicokinetic Study of Orally Administered SCH 58235 In Pregnant Rabbits (SN 99291): **Observations and Measurements**	
Investigation	Performed
Clinical Observations	Daily
Body Weight	Gestation Days 0, 7, 10, 13, 16, and 19
Plasma Analysis for SCH 58235	Gestation Day 19: 1, 2, 4, 6, 12, and 24 hours postdose
Pregnancy Status	At necropsy on Gestation Day 20

Results:

Mortality: One MD female was found dead on GD9. This animal had its thoracic cavity filled with blood, its lungs had dark red focal discoloration and foamy exudate in trachea. The necropsy findings in the dead animal were suggestive of pneumonia and may have been due to misdosing, this was considered incidental by the sponsor.

Clinical signs: No stool, reduced number of fecal pellets, small fecal pellets or fur pulling were observed in all groups, including controls.

Body weight/weight gains: unremarkable in all groups

Food consumption: No drug related effects on food consumption were observed.

Toxicokinetics: This was a separate study as mentioned above. The plasma AUC values are shown in the Table. There was extensive glucuronidation of the drug in rabbits. Exposure to the unconjugated or free drug was less than 0.06% of the total exposure. The AUC values did not proportionally increase with the doses. The AUC of parent + metabolite at 250, 500, 1000 mg/kg/day were 71.5, 95.7, 113.1 μg.h/ml respectively on day 19 of gestation in rats

Tk data on gestation day 19 in segment II study in rabbits

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Toxicokinetic Parameters for Unconjugated SCH 58235 on Day 19 after Oral (Gavage) Administration of SCH 58235 in Pregnant Rabbits			
Dose (mg/kg)	Toxicokinetic Parameters		
	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) (ng.hr/mL)
	SCH 58235		
250	3.68	5.33	44.8
500	3.28	5.50	58.1
1000	4.10	3.75	72.4

Exposure of Rabbits to Conjugated SCH 58235 on Day 19 after Oral (Gavage) Administration of SCH 58235 in Pregnant Rabbits			
Dose (mg/kg)	Toxicokinetic Parameters		
	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) (ng.hr/mL)
	SCH 58235		
250	5096	4.00	71469
500	6171	1.75	95623
1000	6523	1.75	112919

Toxicokinetic Parameters for Total (Unconjugated plus Conjugated) SCH 58235 on Day 19 after Oral (Gavage) Administration of SCH 58235 in Pregnant Rabbits			
Dose (mg/kg)	Toxicokinetic Parameters		
	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) (ng.hr/mL)
	SCH 58235		
250	5098	4.00	71513
500	6174	1.75	95682
1000	6526	1.75	113094

Terminal and necroscopic evaluations: No drug related necropsy observations were seen in mothers.

Dams: No significant effects on pregnancy rates (19/20, 19/20, 20/20, 17/20 were pregnant), sex distribution, distribution in uterus, effects on corpora lutea, implantations were observed. Sponsor states that sex distribution at birth was not effected and was as follows: male live fetuses; 48.8, 48.7, 54.5, 58.6% respectively, female live fetuses; 51.2, 51.3, 45.5, 41.4% respectively, but at MD and HD these ratios may be effected (ratios of M/F at MD and HD were higher i.e. 55/46 and 59/41 respectively vs 49/51 in controls). Mean resorptions were increased at 1000 mg/kg/day (0.37, 0.42, 0.47, 0.82 at 0, 250, 500, 1000 mg/kg/day or 4.1%, 5%, 5.1%, 9.9% respectively). Percent of animals with resorptions were not increased (26.3, 31.6, 30, 29.4% respectively). Sponsor states that half of resorptions occurred in one rabbit (# 71), and were not drug related

Fetal Gross observations: **Following malformations were noted in fetuses:**
Exencephaly and agenesis of tail in one fetus and litter at 250 mg/kg/day.
Head malformed in one fetus and litter at 500 mg/kg/day
Omphalocele (i.e intestinal and viscera protruding) in 1, 3, 1, 2 fetuses and in 1, 3, 1, 2 litters at 0, 250, 500, 1000 mg/kg/day, see Table below on gross observations.
Shortened tail in one fetus and litter at 1000 mg/kg/day.

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Table: Summary of fetal gross observations:

Table 4
ORAL EMBRYO-FETAL DEVELOPMENT STUDY OF SCH 58235 IN RABBITS
SUMMARY OF FETAL GROSS OBSERVATIONS : TOTAL (PERCENT)
SCHEDULED C-SECTION

9638

DOSE GROUP	0 MG/KG	250 MG/KG	500 MG/KG	1000 MG/KG
LITTERS EVALUATED	19	19	19	17
FETUSES EVALUATED	165	152	167	120
LIVE	163	152	167	120
DEAD	2	0	0	0
M EXENCEPHALY				
FETAL INCIDENCE	0	1(0.7)	0	0
LITTER INCIDENCE	0	1(5.3)	0	0
M HEAD MALFORMED				
FETAL INCIDENCE	0	0	1(0.6)	0
LITTER INCIDENCE	0	0	1(5.3)	0
M OMPHALOCELE (MIDLINE DEFECT,INTEST. AND VISCERA PROTUDING)				
FETAL INCIDENCE	1(0.6)	3(2.0)	1(0.6)	2(1.6)
LITTER INCIDENCE	1(5.3)	3(15.8)	1(5.3)	2(11.8)
M AGENESIS OF TAIL				
FETAL INCIDENCE	0	1(0.7)	0	0
LITTER INCIDENCE	0	1(5.3)	0	0
V SHORTENED TAIL				
FETAL INCIDENCE	0	0	0	1(0.8)
LITTER INCIDENCE	0	0	0	1(5.9)
TOTAL FETAL GROSS OBSERVATIONS +				
FETAL INCIDENCE	1(0.6)	4(2.6)	2(1.2)	3(2.5)
LITTER INCIDENCE	1(5.3)	4(21.1)	2(10.5)	3(17.6)

M-MALFORMATION V-VARIATION
+ THE TOTAL REPRESENTS THE TOTAL NUMBER OF OFFSPRING WITH OBSERVATIONS.
THIS NUMBER MAY BE LESS THAN THE SUM OF ALL FINDINGS AS SOME OFFSPRING MAY HAVE MULTIPLE FINDINGS

Skeletal observations: Reduced ossification in parietals and frontal were observed, see Table below. Also, extra pair of thoracic ribs were increased with all doses of the drug (fetal incidences 67, 107, 121, 90, litter incidences 14, 19, 18, 16 respectively), focal thickening of ribs was increased at mid/high doses (fetal/litter incidences 0, 0, 2, 1), distal humeral epiphysis unossified was higher at mid dose (fetal 1, 0, 4, 0, litter 1, 0, 2, 0), scoliosis was observed in 1 fetus/litter at a high dose (fetal incidences 0.8, litter incidences 5.9 vs none in other groups).

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Table Summary of skeletal observations

Table 6
ORAL EMBRYO-FETAL DEVELOPMENT STUDY OF SCH 58235 IN RABBITS
SUMMARY OF SKELETAL OBSERVATIONS : TOTAL (PERCENT)
HYSTERECTOMY C-SECTION

DOSE GROUP	0 MG/KG	250 MG/KG	500 MG/KG	1000 MG/KG
LITTERS EVALUATED	19	19	19	17
FETUSES EVALUATED	163	152	167	128
LIVE	163	152	167	128
DEAD	2	0	0	0
V PARIETALS, REDUCED OSSIFICATION				
FETAL INCIDENCE	0	3(2.0)	5(3.0)	0
LITTER INCIDENCE	0	2(10.5)	2(10.5)	0
V WAVEY OSSIFICATION OF PARIETALS				
FETAL INCIDENCE	0	0	1(0.6)	0
LITTER INCIDENCE	0	0	1(5.3)	0
V FRONTALS, REDUCED OSSIFICATION				
FETAL INCIDENCE	0	1(0.7)	1(0.6)	0
LITTER INCIDENCE	0	1(5.3)	1(5.3)	0
M REDUCED ZYGOMATIC PROCESS				
FETAL INCIDENCE	0	0	1(0.6)	0
LITTER INCIDENCE	0	0	1(5.3)	0
M SPLIT MANDIBLE				
FETAL INCIDENCE	0	0	1(0.6)	0
LITTER INCIDENCE	0	0	1(5.3)	0
M NASAL, REDUCED OSSIFICATION				
FETAL INCIDENCE	0	1(0.7)	0	0
LITTER INCIDENCE	0	1(5.3)	0	0
V INTERPARIETALS, REDUCED OSSIFICATION				
FETAL INCIDENCE	0	1(0.7)	0	0
LITTER INCIDENCE	0	1(5.3)	0	0
V SUPRAOCCIPITAL, REDUCED OSSIFICATION				
FETAL INCIDENCE	0	1(0.7)	0	0
LITTER INCIDENCE	0	1(5.3)	0	0
M HEMIVERTEBRAE				
FETAL INCIDENCE	1(0.6)	0	0	1(0.8)
LITTER INCIDENCE	1(5.3)	0	0	1(5.9)

M-MALFORMATION V-VARIATION
* THE TOTAL REPRESENTS THE TOTAL NUMBER OF OFFSPRING WITH OBSERVATIONS.
THIS NUMBER MAY BE LESS THAN THE SUM OF ALL FINDINGS AS SOME OFFSPRING MAY HAVE MULTIPLE FINDINGS

Total malformations were 3/165, 4/152, 3/167, 5/128 (or 1.8%, 2.6%, 1.8%, 3.9% at 0, 250, 500, 1000 mg/kg/day respectively). However these are not considered drug related by the sponsor as they were seen in all groups. Similarly visceral/skeletal or gross variations are also not considered drug related.

Summary of Segment II rabbit study with SCH 58235: In a segment II teratology study in rabbits, pregnant animals (n=20/group) were given oral SCH 58235 by gavage at doses of 0, 250, 500, 1000 mg/k/day from day 7 to day 19 of gestation. Females were sacrificed on day 30 PC and necropsied. In females, mean resorptions were increased at 1000 mg/kg/day (9.9% vs 4.1% in controls). At 250-1000 mg/kg/day increased incidence of malformations were observed in fetuses and litters. These included exencephaly (in one fetus and litter at 250 mg/kg/day), agenesis of tail (in one fetus and litter at 250 mg/kg/day), head malformed (in one fetus and litter at 500 mg/kg/day), omphalocele (i.e intestinal and viscera protruding in 1-3 fetuses and litters at low and high doses (1, 3, 1, 2 respectively), and shortened tail in one fetus and litter at 1000 mg/kg/day. Skeletal observations were noted at low & mid doses. These included extra pair of thoracic ribs which were increased at all doses of the drug (fetal incidences 90-107 vs 67 in controls, litter incidences 16-19 vs 14 in controls), reduced ossifications in

parietals (fetal incidences 0, 3, 5, 0, litter incidences 0, 2, 2, 0) and frontals (fetal/litter incidences 0, 1, 1, 0). Increased focal thickening of ribs at mid/high doses (fetal/litter incidences 0, 0, 2, 1), and increased unossified distal humeral epiphysis at a mid dose (fetal 1, 0, 4, 0, litter 1, 0, 2, 0). Scoliosis was observed in 1 fetus/litter at a high dose (fetal incidences 0.8, litter incidences 5.9 vs none in other groups). Scoliosis (HD) and increased focal thickening of ribs (MD & HD), both may be dose related. Also MD & HD produced increased male fetuses compared to female fetuses. The historical control means for malfomations have not been provided by the sponsor.
The AUC values of the total drug (parent + metabolite) at maternal NOAEL of 500 mg/kg/day in rabbits on gestation day 19 (95.7 µg.h/ml) was 140-fold, the human AUC at 10 mg/day (0.68 µg.h/ml). The AUC values of the total drug at embryo-fetal NOAEL of <250 mg/kg/day in rabbits (71.5 µg.h/ml) was <100-fold, the human AUC at 10 mg/day (0.68 µg.h/ml).

Maternal NOAEL was 500 mg/kg/day in segment II study in rabbits (or 140-fold the human dose of 10 mg/day based on exposures, and ≈ 1000-fold the human dose of 10 mg/day based on body surface area), as increased resorptions were observed at 1000 mg/kg/day. **The embryo-fetal NOAEL was <250 mg/kg/day** (or <100-fold the human dose of 10 mg/day based on exposures, and ≈ 500-fold the human dose of 10 mg/day based on body surface area), since developmental toxicity (increased malformations in tails, head and in visceral area, and skeletal variations) was noted from the lowest dose of 250 mg/kg/day. Sponsor's NOAEL's are 1000 mg/kg/day in segment II study in rabbits for both maternal and embryo fetal toxicity.

4. Segment II toxicokinetics (TK) study in rabbits and fetuses (study # 01022). Study title: study of placental transfer of SCH 58235 when administered orally by gavage to rabbits (vol 1.186, page 1, reference 18, study # 01022)

The object of this study was to examine the maternal systemic exposure and placental transfer of the drug into the fetus in rabbits. The drug was given to rabbits (NZW) during gestation day 7 through gestation day 22. The drug was given only at one high dose of 1000 mg/kg/day by gavage (total n= 30, n=3/time point). Drug batch Number was 97-58235-X-02.

Maternal blood samples were collected from 15 rabbits after gestation day 10, and from 15 rabbits after gestation day 22 (at 1, 2, 4, 8, 24 hrs). From day 5, clinical signs were examined and body weights on day 0 and from day 7 to day 22. This study was mostly designed to look at the PK of the drug during pregnancy in maternal and fetal plasma.

Study design for TK study in segment III in rabbits

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Study of Placental Transfer of SCH 58235 when Administered Orally by Gavage to Rabbits (SN 01022): **Study Design**				
Group	Total Daily Dose (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Number of Females[a]
Group 1 (SCH 58235)	1000	5	200	30
a: Maternal blood samples (one time point per animal) were collected from fifteen rabbits after the gestation Day 10 dose and fifteen rabbits after the gestation Day 22 dose as follows. Following dosing on gestation Day 10 or 22, maternal and fetal blood (gestation Day 22 only) was collected at 1, 2, 4, 8 and 24 hours (3 rabbits or litters/time point). Additionally, whole live embryos (gestation Day 10) or placentas and fetal livers (gestation Day 22) were collected and retained for possible SCH 58235 analysis.				

Parameters and end points evaluated

Study of Placental Transfer of SCH 58235 when Administered Orally by Gavage to Rabbits (SN 01022): **Observations and Measurements**	
Investigation	Performed
Viability[a]	Daily
Maternal Blood Collection for SCH 58235 Analysis	Gestation Day 10 and 22 (1, 2, 4, 8 and 24 hrs after dosing
Fetal Blood Collection for SCH 58235	Gestation Day 22 (following the maternal blood collection for each dam)
Collection of Embryo/Fetal Tissue for Possible SCH 58235 Analysis[b]	Gestation Day 10: Embryos Gestation Day 22: Fetal Placenta and Livers
Necropsy/C-Section[c]	Gestation Day 10 or 22 (following the maternal blood collection for each dam)
Reproductive Parameters[d]	Yes
a: Remarkable clinical observations were also recorded. Additionally, body weights were measured only for the purpose of dose administration. b: Following maternal and fetal (when applicable) blood collections. c: Examined to confirm pregnancy status only. d: Only the total number of live embryos/fetuses was recorded.	

Results:

Mortality: Two rabbits died, one on gestation day 16 (died shortly after dosing, so necropsy was performed) and the other on GD-17 (necropsy showed white material in the thoracic cavity adhered to all lobes of the right lung and the thoracic wall). Since no deaths were noted in the standard segment II study in rabbits these were considered incidental by the sponsor and not related to the drug. However, one rabbit did died in a standard segment II study in rabbits at 500 mg/kg/day and this also had its thoracic cavity filled with blood and had dark red focal discoloration and foamy exudate in trachea. The pregnancy status in above two dead animals was not determined.

During termination of the study it was observed that total of 3/30 rabbits were not pregnant. One rabbit on day 10 in to the study was not pregnant. Two rabbits on days 22-23 in to the study were not pregnant. We do not know if they were excluded from the TK analysis or not.

Toxicokinetics: The plasma conc. and AUC values are shown in the Table. The systemic exposures of the total drug was higher on gestation day 10 (180.7 µg.h/ml), vs day 22 (157.9 µg.h/ml), while in fetuses the exposure of the total drug was much lower

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on gestation days 22 (4.7 µg.h/ml). The palsma conc of the total drug in mother on day 10 and 22 were 9.8 & 9.4 µg/ml respectively, and in fetus it was much lower than the mothers on day 22 of gestation (0.3 µg.h/ml). The fetal:dam exposure ratio on gestation day 22 to total and conjugated drug was 0.03, while to the unconjugated drug fetal exposure was higher than in the mother, and was 1.38. Extensive glucuronidation of the drug was noted in mothers on gestation day 10-22 and in the fetus on gestation day 22 (the percent total conjugated drug accounted for >99% and >96% of total in dams and fetuses). In another rabbit study where the drug was given to rabbits on GD 7 through GD 19, the systemic exposures (AUC 0-24 hr) were 113, 113, and 0.073 µg.h/ml for the total, unconjugated and conjugated drug respectively, and these values are slightly lower (25-45% lower) than the current study. This is because serial sampling was done at different times

Table A. Exposures of the drug in mothers and fetuses in segment II in rabbits

Results: The systemic exposure to SCH 58235 [AUC(0-24 hr)] is summarized in the following table:

	AUC(0-24 hr) (ng-hr/mL)[a]		
	Total SCH 58235	Conjugated SCH 58235	Unconjugated SCH 58235
Dam GD[b] 10	180702	180574	129
Dam GD 22	157923	157801	122
Fetus GD 22	4685	4517	169

a: Calculated from mean composite data from one to three rabbits per time point.
b: GD = Gestation Day

Table B. Exposures of the drug in mothers (on gestation days 10, and 22) and fetuses (on gestation day 22) in segment II in rabbits

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Table 1 Toxicokinetic Parameters of Total, Conjugated, and Unconjugated SCH 58235 on Gestation Days 10 and 22 Following Multiple Oral Gavage Administration (Gestation Day 7 through Gestation Day 22) of 1000 mg/kg SCH 58235 to Female Rabbits

Parameter	Units	Total SCH 58235		
		Dam GD 10	Dam GD 22	Fetus GD 22
Cmax	ng/mL	9809	9405	294
Tmax	hr	24	1	24
AUC(0-24 hr)	ng·hr/mL	180702	157923	4685
Exposure Ratio		–	0.87[a]	0.03[b]
		Conjugated SCH 58235[c]		
		Dam GD 10	Dam GD 22	Fetus GD 22
Cmax	ng/mL	9801	9401	286
Tmax	hr	24	1	24
AUC(0-24 hr)	ng·hr/mL	180574	157801	4517
% Total[d]	%	99.9	99.9	96.4
Exposure Ratio		–	0.87[a]	0.03[b]
		Unconjugated SCH 58235		
		Dam GD 10	Dam GD 22	Fetus GD 22
Cmax	ng/mL	7.61	6.84	7.92
Tmax	hr	24	24	24
AUC(0-24 hr)	ng·hr/mL	129	122	169
% Total[e]	%	0.1	0.1	3.6
Exposure Ratio		–	0.95[a]	1.38[b]

a: Calculated as Dam [Gestation Day 22 AUC(0-24 hr) ÷ Gestation Day 10 AUC(0-24 hr)]

b: Calculated as Gestation Day 22 [Fetal AUC(0-24 hr) ÷ Dam AUC(0-24 hr)]

c: Calculated as plasma total SCH 58235 minus plasma unconjugated SCH 58235, and reported as SCH 58235 equivalents.

d: Calculated as [conjugated SCH 58235 AUC(0-24 hr) ÷ total SCH 58235 AUC(0-24 hr)]*100

e: Calculated as [unconjugated SCH 58235 AUC(0-24 hr) ÷ total SCH 58235 AUC(0-24 hr)]*100

In summary, the drug crosses the placenta when given to pregnant rabbits on GD 7-GD 22 Exposure to the conjugated accounted for majority of the drug in dams and pups, the percent total for conjugated drug was 99% and 96% in dams and fetuses respectively. Thus AUC of the total drug (parent + metabolite) at 1000 mg/kg/day on gestation day 10 and 22 were 180.1 & 157.9 μg.h/ml, and in fetus on GD 22 was 4.7 μg.h/ml, see Tables A-B.

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5. Segment III study: Effects of SCH 58235 on pre- and postnatal developmental in rats

Key study findings:
Maternal NOAEL was 1000 mg/kg/day, as 300-1000 mg/kg/day produced slight increase in clinical signs (dried red material around nose in 0/25, 1/25, 5/25, 1/25 respectively, hair loss in 0/25, 0/25, 0/25, 2/25 respectively) and at necropsy findings (hair loss at mid-high doses in 0/23, 0/24, 1/23, 2/24) in mothers. **The NOAEL for pre/post natal toxicity was <100 mg/kg/day**, as mortality was observed in one male on day 155. Clinical signs were observed at mid-high doses in F1 pups (subcutaneous hemorrhage, number of findings/number of pups with findings 0/0, 1/1, 1/1, 4/3, missing tail in 1 pup at a mid dose, uneven hair growth in 0/0, 0/0, 1/1, 2/2 respectively). Post weaning clinical signs were also increased in F1 animals (increased dry material around eyes/ears/nose number of findings/number of pups with findings 1/1, 5/3, 0/0, 3/3 respectively, increased hair loss in left and/or right forelimbs in male (2, 4, 4, 3 respectively) and female rats (4, 8, 11, 8 respectively). Post natal learning and memory developmental tests in F1 rats were normal on day 1 (at all doses) but decreased time intervals in these tests were observed on days 5-7 in males and increased time intervals in these tests on days 3-7 in females at all drug doses. One F1 male died at the lowest dose of 100 mg/kg/day on day 155, this death was attributed to the drug administration in mothers. Scheduled necropsy of F1 rats again showed hair loss in 0/24, 2/22, 0/23, 1/25. In F2 pups, tail was missing in 3 pups at mid doses vs none in other groups, and subcutaneous hemorrhage was higher at mid-high doses, see the text. The maternal NOAEL was 1000 mg/kg/day (or 1000-fold the human dose of 10 mg/day, based on body surface area). The embryo-fetal NOAEL was <100 mg/kg/day (or <100 fold the human dose of 10 mg/day, based on body surface area).

Study no.: 96386
Volume #, and page #: 1.37, pg.1 (reference 26)
Conducting laboratory and location: Safety Evaluation Center/Schering-Plough Research Center; Layfayette, NJ
Date of study initiation: 7/22/1999
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 97-58235-X-02

Formulation/vehicle: 0.4% aq. methylcellulose

Methods:

Species/strain: Rat (Crl:CD(SD)IGS BR
Doses employed: 0, 100, 300, 1000 mg/kg SCH 58235
Route of administration: oral gavage
Study design: 0, 100, 300, 1000 mg/kg SCH 58235 were given to F0 females from gestation day 6 through lactation day 21. Animals necropsied on day 30.

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Study Design:

A Prenatal and Postnatal Developmental Toxicity and Maternal Function Study of SCH 58235 Administered by Oral Gavage in Rats (SN 96386): Study Design				
Group	SCH 58235			
	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	Number of Females
Control (0.4% methylcellulose)	0	5	0	25
Low-Dose (SCH 58235)	100	5	20	25
Mid-Dose (SCH 58235)	300	5	60	25
High-Dose (SCH 58235)	1000	5	200	25

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Parameters/end points evaluated. The timings of neurobehavioral and learning assessments performed are also given in this Table.

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A Prenatal and Postnatal Developmental Toxicity and Maternal Function Study of SCH 58235 Administered by Oral Gavage in Rats (SN 96386): Observations and Measurements			
Investigation	**Performed**	**Investigation**	**Performed**
Clinical Observations (F_0):	Daily from gestation Day 0 until lactation Day 21	Sex Determination (F_1):	Postnatal Days 1, 4 and 21
Food Consumption (F_0):	Gestation Days 0, 6, 9, 12, 15, 18 and 21 and on lactation Days 1, 4, 7, 14 and 21.	Necropsy (F_0):	Postnatal Day 21
		Culling (F_1):	Postnatal Day 4 culled to four per sex/litter (when possible)
Body Weight (F_0):	F_0 females weighed on gestation Days 0, 6, 9, 12, 15, 18, 21 and on lactation Days 1, 4, 7, 14, and 21.	Acoustic Startle (F_1):	Postnatal Days 20 and 60
		Locomotor Activity (F_1):	Postnatal Days 21 and 60
		Learning and Memory Test — Maze, F_1):	Postnatal Days 23 through 29
Dosing Females (F_0):	Once daily from gestation Day 6 through lactation Day 21	Vaginal Opening (F_1):	Beginning on postnatal Day 25 and continuing daily until perforation is present
Parturition (F_0 and F_1)	Twice daily during the period of expected parturition. Length of gestation for each dam calculated.	Balanopreputial Separation (F_1):	Beginning on postnatal Day 35 and continuing daily until separation is present
		Vaginal Cytology (F_0):	Two weeks prior to and during the mating period
Litter Size (F_0):	Daily until lactation Day 21		
Clinical Observations (F_1):	Postnatal Days 1, 4, 7, 14 and 21 and weekly thereafter	Mating (F_1):	91 to 96 days of age
Survival (F_1):	Daily from postnatal Day 0 until postnatal Day 21	Observations (F_2):	Daily from birth until postnatal Day 4. Detailed physical exam, pup body weight and sex on postnatal Days 1 and 4
Body Weights (F_1):	Postnatal Days 1, 4 (prior to culling), 7, 14, and 21 and weekly thereafter until euthanasia for the males or gestation for the females, then on gestation Days 0, 6, 9, 12, 15, 18, 21 and on lactation Days 1 and 4.	Male Necropsies (F_1):	At 20-21 weeks of age (following termination of F_2 offspring on postnatal Day 4)
		Dam Necropsies (F_1) and Pup Euthanasia (F_2):	Lactation Day 4

Toxicokinetics: A separate study was conducted to assess the systemic exposure of the drug in Segment III study in rats (vol 1.184, page 1, reference 16, study # 01023). The

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drug was given only at the highest dose of 1000 mg/kg/day by gavage on gestation day 6 through lactation day 12 (total n=3/time point). Drug batch Number was 97-58235-X-02.

Study design for TK study in segment III in rats

Study of Placental and Lactation Transfer of SCH 58235 When Administered Orally by Gavage to Rats (SN 01023): Study Design				
Group	Total Daily Dose (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Number of Females[a]
Group 1 (SCH 58235)	1000	5	200	48
a: A total of three rats/time point were assessed at 1, 2, 4, 8 and 24 hours post-dose for placental (gestation Days 10 and 20) and lactation (lactation Day 12) transfer. Three additional dams were assessed at one hour post-dose for lactation transfer (lactation Day 12)				

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Parameters and end points evaluated in the TK study in rats

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Study of Placental and Lactation Transfer of SCH 58235 When Administered Orally by Gavage to Rats (SN 01023): **Observations and Measurements**	
Investigation	Performed
Viability[a]	Daily
Litter Size	Yes
Maternal Plasma Analysis for SCH 58235	Gestation Days 10 or 20, or lactation Day 12 (1, 2, 4, 8 and 24 hrs after dosing)
Maternal Milk Analysis for SCH 58235[b]	Lactation Day 12[c]
Fetal Plasma Analysis for SCH 58235	Gestation Day 20
Fetal Tissue Analysis for SCH 58235[b]	Gestation Day 10: Embryos with visceral yolk sac Gestation Day 20: Placenta and liver
Pup Plasma Analysis for SCH 58235	Lactation Day 12[d]
Necropsy[e]	Yes
Reproductive Parameters[f]	Yes

a: Clinical observations were performed when needed, only for the purpose of monitoring the general health of the dam and are presented in an appendix to the final report. Additionally, body weights were measured only for the purpose of dose administration and are also presented in an appendix to the final report.

b: Tissue (embryo, placenta and fetal liver) and milk samples were collected but not analyzed.

c: Maternal milk was collected within 15 minutes prior to maternal blood collection on lactation Day 12. Dams scheduled for milk collection at the 1 and 2 hr time points post-dose were separated from their pups at the time of dosing. Dams scheduled for milk collection at the 4, 8 and 24 hr time points were separated from their pups two hours prior to sample collection.

d: Pups from the dams designated for the 1- or 2-hour milk collection time points were not sampled for plasma analysis. Pups from the dams designated for the 4, 8 and 24 hr milk collection time points were sacrificed and plasma samples were collected at the time the dam was separated from the litter (two hours prior to milk collection). Pups from additional dams (designated only for plasma analysis at the 1-hour time point) were sacrificed and plasma samples were collected at the 1-hour time point.

e: Dams sacrificed during gestation were examined to confirm pregnancy status only. No further necropsy examinations were performed.

f: Only the total number of live embryos/fetuses was recorded.

Mortality and Clinical signs: No mortality was observed. Clinical signs such as dried red material on right forearm (0/0, 2/2, 2/1, 0/0), dried red material around nose (in 0/25, 1/25, 5/25, 1/25 respectively) was observed at low-mid or at mid doses, whereas hair loss (total occurences/number of animals 0/0, 0/0, 0/0, 29/2), and dried red material around nose (0/0, 1/1, 8/5,1/1) was increased at a mid dose.

Body weight: No effects on body weights during gestation or during 0-21 days of lactation were observed.

Food consumption: No significant effects during gestation or lactation were observed.

Toxicokinetics: As mentioned earlier,TK was conducted in a separate study. There was no drug related mortality in this study (# 01023), there were 16 litters with 12-16 pups/litter. The plasma conc. and AUC values are shown in the Table. The systemic exposures of the drug was higher on gestation days 10 (5.8 µg.h/ml), than on day 20 (12.2 µg.h/ml) and this was further increased in fetuses on gestation days 20 (18.7 µg.h/ml). The plasma conc in mother on day 10 and 20 were 0.37 & 1.1 µg/ml respectively, and in fetus it was two fold higher than the mothers on day 20 of gestation (2.0 µg.h/ml). On gestation day 20, exposure to total (by 1.5 fold) and conjugated drug (by 2.2 fold) was higher in fetus than in the mother, while to the unconjugated drug fetal

exposure was lower than in the mother. Extensive glucuronidation of the drug was noted in mothers on gestation day 10-20 and in the fetus on gestation day 20 (the percent total conjugation in mothers on day 10 and 20 was 99%, 66% and in the fetus 94% respectively). The drug was transferred to lactating rats, the exposures on lactation day 12 in mothers were 23.1 µg.h/ml , and in nursing pups 11.3 µg.h/ml respectively . However exposures in nursing pups were lower than in lactating rats (the ratios were 0.5, 0.7, and 0.016 for total, free and conjugated drug). Exposure to the conjugated accounted for majority of the drug in lactating dams and pups, the percent total for conjugated drug was 72% and 99% in dams and pups respectively.
Thus AUC of the total drug (parent + metabolite) at 1000 mg/kg/day on gestation day 10 and 20 were 5.8 & 12.2 µg.h/ml, and in fetus on GD 20 was 18.7 µg.h/ml. These values on lactation day 12 in dams and pups were 23.1 & 11.3 µg.h/ml, see Tables A-B.

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Table A. TK parameters in mothers and fetuses during gestation

Table 1 Toxicokinetic Parameters of Total, Conjugated and Unconjugated SCH 58235 on Gestation Day 10 and 20 Following Multiple Oral (Gavage) Administration (Gestation Day 6 through Gestation Day 10 or 20) of 1000 mg/kg SCH 58235 to Female Rats

		Total SCH 58235		
		Dam GD 10	Dam GD 20	Fetus GD 20
Cmax	ng/mL	369	1090	2014
Tmax	hr	2	24	24
AUC(0-24 hr)	ng·hr/mL	5775	12208	18730
Fetal:Dam[a]		NA	NA	1.53
		Conjugated SCH 58235[b]		
		Dam GD 10	Dam GD 20	Fetus GD 20
Cmax	ng/mL	366	675	1899
Tmax	hr	2	24	24
AUC(0-24 hr)	ng·hr/mL	5697	8060	17531
% Total[c]	%	99	66	94
Fetal:Dam[a]		NA	NA	2.18
		Unconjugated SCH 58235		
		Dam GD 10	Dam GD 20	Fetus GD 20
Cmax	ng/mL	4.04	415	114
Tmax	hr	8	24	24
AUC(0-24 hr)	ng·hr/mL	79.1	4149	1219
% Total[d]	%	1	34	7
Fetal:Dam[a]		NA	NA	0.294

a: Calculated as Gestation Day 20 [Fetal AUC(0-24 hr) + Dam AUC(0-24 hr)]

b: Calculated as plasma total SCH 58235 minus plasma unconjugated SCH 58235, and reported as SCH 58235 equivalents.

c: Calculated as [conjugated SCH 58235 AUC(0-24 hr) ÷ total SCH 58235 AUC(0-24 hr)]*100

d: Calculated as [unconjugated SCH 58235 AUC(0-24 hr) ÷ total SCH 58235 AUC(0-24 hr)]*100

Table B. TK parameters in mothers and fetuses during lactation

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Table 2 Toxicokinetic Parameters of Total, Conjugated and Unconjugated SCH 58235 on Lactation Day 12 Following Multiple Oral (Gavage) Administration (Gestation Day 6 through Lactation Day 12) of 1000 mg/kg SCH 58235 to Female Rats

		Total SCH 58235	
		Dam LD 12	Pup LD 12
Cmax	ng/mL	1471	750
Tmax	hr	2	22
tf	hr	24	22
AUC(tf)	ng-hr/mL	23111	11291
Pup:Dam[a]		NA	0.489
		Conjugated SCH 58235[b]	
		Dam LD 12	Pup LD 12
Cmax	ng/mL	1067	742
Tmax	hr	1	22
tf	hr	24	22
AUC(tf)	ng-hr/mL	16716	11191
% Total[c]	%	72	99
Pup:Dam		NA	0.669
		Unconjugated SCH 58235	
		Dam LD 12	Pup LD 12
Cmax	ng/mL	1037	7.36
Tmax	hr	2	22
tf	hr	24	22
AUC(tf)	ng-hr/mL	6396	101
% Total[d]	%	28	1
Pup:Dam		NA	0.0158

a: Calculated as LD [Pup AUC(tf) ÷ Dam AUC(tf)]

b: Calculated as plasma total SCH 58235 minus plasma unconjugated SCH 58235, and reported as SCH 58235 equivalents.

c: Calculated as [conjugated SCH 58235 AUC(tf) ÷ total SCH 58235 AUC(tf)]*100

d: Calculated as [unconjugated SCH 58235 AUC(tf) ÷ total SCH 58235 AUC(tf)]*100

Maternal necropsy findings: Red matting in skin (0/23, 1/24, 1/23, 0/24 respectively) was noted at low-mid doses, and increased skin hair loss (0/23, 0/24, 1/23, 2/24 respectively) at mid-high doses in rats.

Reproduction Parameters and Embryonic Development in F0 Rats: Gestation length was not altered compared to controls (Mean 21.7, 21.6, 21.7, 21.6 respectively), the animals which failed to deliver were also comparable between groups (2, 1, 2, 1 respectively), these females were internally normal. Necropsy on rest of the females on lactation day 21 showed that all females were internally normal. No effects on litter size and fetal sex distribution were noted at any dose. No effects on pup survival were observed.

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Pre-weaning clinical signs in F1 animals: Body weights were not significantly different from controls. However, tail was missing in 1 pup at a mid dose, subcutaneous hemorrhage was noted at all doses (0, 1, 1, 3 respectively), uneven hair growth was noted at mid/high doses (0, 0, 1, 2 respectively) and animals small in size were observed at all doses (0, 2, 1, 4 respectively).

Table. Clinical signs in F1 pups

TABLE 34 - F1 GENERATION

PROJECT NO.:WIL-370011 — PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS — PAGE 1

SPONSOR:SCHERING-PLOUGH — SUMMARY OF PUP CLINICAL OBSERVATIONS

SPONSOR NO.:96386

PRE-WEANING

TOTAL NUMBER OF FINDINGS/NUMBER OF PUPS WITH FINDING

FINDING	GROUP : 1	2	3	4
NORMAL				
NO REMARKABLE OBSERVATIONS	1241/352	1285/358	1240/352	1266/353
DISPOSITION				
FOUND DEAD	17/ 17	6/ 6-A	9/ 9	4/ 4
EUTHANIZED IN EXTREMIS	1/ 1	0/ 0	2/ 2	0/ 0
SCHEDULED EUTHANASIA (PND 21)	130/130	142/142	131/131	140/140
CULLED ON SCHEDULED DAY (PND 4)	168/168	164/164	167/167	156/156
MISSING	7/ 7	6/ 6	10/ 10	9/ 9
BODY/INTEGUMENT				
MISSING TAIL	0/ 0	0/ 0	1/ 1	0/ 0
SUBCUTANEOUS HEMORRHAGE(S)	0/ 0	1/ 1	1/ 1	4/ 3
DISTAL TAIL BLACK IN COLOR	2/ 1	2/ 1	0/ 0	2/ 1
SMALL IN SIZE	0/ 0	2/ 2	2/ 1	4/ 4
SCABBING	1/ 1	0/ 0	0/ 0	0/ 0
UNEVEN HAIR GROWTH	0/ 0	0/ 0	1/ 1	2/ 2
DORSAL TAIL BLACK IN COLOR	1/ 1	0/ 0	0/ 0	0/ 0
CARDIO-PULMONARY				
SHALLOW RESPIRATION	1/ 1	1/ 1	1/ 1	0/ 0

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

A = PUP NO. 25822-01 WAS DETERMINED TO HAVE SPINA BIFIDA AT NECROPSY (SEE TABLES 40 AND 41)

In pups that were found dead, their necropsies (in 19, 6, 11, 4 pups respectively) showed malformations of spina bifida in 1 fetus and 1 litter at 100 mg/kg/day and this was considered incidental.

Table: Necropsies in dead pups

TABLE 40 - F1 GENERATION

PROJECT NO.:WIL-370011 — PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS

SPONSOR:SCHERING-PLOUGH — SUMMARY OF PUP NECROPSY FINDINGS

SPONSOR NO.:96386 — FOUND DEAD PUPS

DOSE GROUP:	PUPS 1	2	3	4	LITTERS 1	2	3	4
NUMBER EXAMINED VISCERALLY	19	6	11	4	7	5	5	4
STOMACH								
MILK NOT PRESENT-A	9	2	7	0	5	2	4	0
MILK PRESENT-A	1	1	1	1	1	1	1	1
MALFORMATION								
SPINA BIFIDA	0	1	0	0	0	1	0	0

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

A = PRESENCE OR ABSENCE OF MILK DETERMINED FOR PUPS FOUND DEAD ON PND 0 OR PND 1

Necropsies in surplus pups (culled) on PND-4 showed major blood vessel variations in LD< MD all groups including controls (in 1 fetus and 1 litter each), and small kidney at low dose in 1 fetus and 1 litter vs none in other groups.

Table: Necropsies in culled pups on day 21:

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TABLE 42 (CULLED PUPS) - F1 GENERATION
PROJECT NO.:WIL-370011 PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
SPONSOR:SCHERING-PLOUGH SUMMARY OF PUP NECROPSY FINDINGS
SPONSOR NO.:96386 CULLED PUPS

DOSE GROUP:	PUPS 1	2	3	4	LITTERS 1	2	3	4
NUMBER EXAMINED VISCERALLY	168	164	167	156	22	23	22	24
VARIATION								
KIDNEY(S) - SMALL	0	1	0	0	0	1	0	0
MAJOR BLOOD VESSEL VARIATION	1	1	1	0	1	1	1	0

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

Table: Necropsies in euthanized pups

TABLE 44 (SURPLUS PUPS) - F1 GENERATION
PROJECT NO.:WIL-370011 PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
SPONSOR:SCHERING-PLOUGH SUMMARY OF PUP NECROPSY FINDINGS
SPONSOR NO.:96386 EUTHANIZED PUPS

DOSE GROUP:	PUPS 1	2	3	4	LITTERS 1	2	3	4
NUMBER EXAMINED VISCERALLY	130	142	131	140	23	24	23	24
KIDNEY								
DILATED PELVIS	0	1	1	0	0	1	1	0
URETER								
DISTENDED	0	0	1	0	0	0	1	0

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

Post-natal development of F1 pups: The effects on neurobehavioral/developmental parameters in pups were examined. The acoustic startle response on PND 20 and PND 60 (which included peak amplitude (V_{max}), latency to peak response (T_{max}) and the average response (V_{ave}) were not significantly different from controls. Locomotor activity (total and ambulatory activity), balanoprepetual separation (the mean day of acquisition and mean body weights on the day of balanoprepetual separation), and vaginal patency (vaginal opening, and the mean day of acquisition and mean body weights on the day of vaginal patency) were all uneffected and were similar to controls. Similarly learning and memory test (— maze swimming test which show mean time to escape the straight channel, the mean number of errors, and the overall probe) did not show drug related effects on learning and memory on testing day 1 in both sexes. However in — maze test in males on testing day 5 (trial 8, in path-B 93.6, 42.6, 72.9, 80.7 sec at 0, 100, 300, 1000 mg/kg/day) and on testing day 7 (trial 12, in path-A 72.8, 50.6, 44.5, 63.9 sec respectively), the mean time was decreased in all groups. While, in females these times were increased on day 3 to day 7 (trial 4 path A 29, 60, 49, 42 sec respectively, trial 5 path B 140, 135, 165, 157 sec respectively, trial 6 path B 106, 122, 132, 143 sec respectively), See Tables A-D. However sponsor states that no drug related effects on learning or memory tests were observed on testing day 1 and in the mean number of errors and the overall probe (trial 11 and 12) compared to controls.

Tables A-D. Postnatal development in F1 rats

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Table A. . Postnatal development in F1 male rats

TABLE 5@ (PHD 23-291- F1 GENERATION
PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
— MAZE SWIMMING TRIALS - GROUP SUMMARY
MALES

PROJECT NO.:WIL-370011
SPONSOR:SCHERING-PLOUGH
SPONSOR NO.:96186

PAGE 3

GROUP:	1	2	3	4
TRIAL 8 (DAY 5) - PATH B				
MEAN TIME (SECONDS)	91.60	42.63	72.85	80.72
S.D.	74.807	27.236	57.160	72.915
MEAN NO. ERRORS	16	6	9	13
S.D.	16.9	4.4	7.3	13.0
N	10	10	10	10
TRIAL 9 (DAY 6) - PATH B				
MEAN TIME (SECONDS)	82.24	89.98	78.48	103.17
S.D.	62.606	78.186	62.656	70.909
MEAN NO. ERRORS	15	14	14	18
S.D.	14.3	13.6	13.4	14.0
N	10	10	10	10
TRIAL 10 (DAY 6) - PATH B				
MEAN TIME (SECONDS)	63.42	62.28	61.22	58.90
S.D.	69.253	56.727	51.654	57.432
MEAN NO. ERRORS	10	10	14	9
S.D.	12.9	11.4	13.3	8.5
N	10	10	10	10
TRIAL 11 (DAY 7) - PATH A (PROBE)				
MEAN TIME (SECONDS)	63.51	91.67	62.84	81.83
S.D.	51.383	65.267	26.238	51.694
MEAN NO. ERRORS	12	21	16	17
S.D.	9.5	17.7	7.2	15.8
N	10	10	10	10

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

A = FORWARD ROUTE THROUGH MAZE
B = REVERSE ROUTE THROUGH MAZE

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Table B. Postnatal development in F1 male rats

TABLE 58 (PND 23-29)- F1 GENERATION
PROJECT NO.:WIL-370011 — PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS — PAGE 4
SPONSOR:SCHERING-PLOUGH — MAZE SWIMMING TRIALS - GROUP SUMMARY
SPONSOR NO.:96386 — MALES

GROUP:	1	2	3	4
TRIAL 12 (DAY 7) - PATH A (PROBE)				
MEAN TIME (SECONDS)	72.81	50.60	44.45	63.97
S.D.	38.885	41.718	25.599	42.008
MEAN NO. ERRORS	14	11	8	14
S.D.	8.7	14.0	6.4	11.6
N	10	10	10	10
OVERALL — (TRIALS 1-10)				
MEAN TIME (SECONDS)	83.41	79.62	81.25	88.19
S.D.	27.145	30.112	23.203	26.276
MEAN NO. ERRORS	14	14	15	15
S.D.	4.7	6.8	4.6	4.9
N	10	10	10	10
OVERALL PROBE (TRIALS 11-12)				
MEAN TIME (SECONDS)	68.16	73.14	53.65	72.98
S.D.	38.676	48.755	28.679	39.818
MEAN NO. ERRORS	13	16	11	16
S.D.	6.9	14.2	5.2	11.7
N	10	10	10	10

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

A - FORWARD ROUTE THROUGH MAZE
B - REVERSE ROUTE THROUGH MAZE

None significantly different from the control group

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Table C. Postnatal development in F1 female rats

TABLE 58 (PND 23-39)- F1 GENERATION
PROJECT NO.:WIL-170011 — PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS — PAGE 6
SPONSOR:SCHERING-PLOUGH — MAZE SWIMMING TRIALS - GROUP SUMMARY
SPONSOR NO.:96386 — FEMALES

GROUP:	1	2	3	4
TRIAL 4 (DAY 3) - PATH A				
MEAN TIME (SECONDS)	38.75	60.18	48.77	42.87
S.D.	11.295	31.922	33.085	21.105
MEAN NO. ERRORS	6	12	10	7
S.D.	3.9	8.0	9.5	3.8
N	10	10	10	10
TRIAL 5 (DAY 4) - PATH B				
MEAN TIME (SECONDS)	139.50	134.90	165.27	157.04
S.D.	62.911	54.692	24.911	51.306
MEAN NO. ERRORS	24	24	31	25
S.D.	13.2	16.5	9.2	12.1
N	10	10	10	10
TRIAL 6 (DAY 4) - PATH B				
MEAN TIME (SECONDS)	105.96	122.20	132.01	142.87
S.D.	65.750	64.662	65.799	67.962
MEAN NO. ERRORS	21	21	24	22
S.D.	14.9	14.3	12.3	12.0
N	10	10	10	10
TRIAL 7 (DAY 5) - PATH B				
MEAN TIME (SECONDS)	121.07	109.85	118.27	121.26
S.D.	60.211	66.052	70.219	65.375
MEAN NO. ERRORS	22	17	16	19
S.D.	11.8	12.4	11.3	10.5
N	10	10	10	10

1- 8 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

A = FORWARD ROUTE THROUGH MAZE
B = REVERSE ROUTE THROUGH MAZE

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Table D. Postnatal development in F1 female rats

TABLE 58 (PND 23-29)- F1 GENERATION
PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
MAZE SWIMMING TRIALS - GROUP SUMMARY
FEMALES

PROJECT NO.:WIL-370011
SPONSOR:SCHERING-PLOUGH
SPONSOR NO.:96386

GROUP:	1	2	3	4
TRIAL 8 (DAY 5) - PATH B				
MEAN TIME (SECONDS)	65.48	89.67	90.93	116.03
S.D.	61.799	63.583	60.820	56.646
MEAN NO. ERRORS	14	17	15	19
S.D.	17.8	15.8	18.2	9.6
N	10	10	10	10
TRIAL 9 (DAY 6) - PATH B				
MEAN TIME (SECONDS)	59.87	83.82	116.86	101.38
S.D.	38.496	72.177	65.583	75.529
MEAN NO. ERRORS	11	10	20	17
S.D.	9.5	9.9	13.6	14.5
N	10	10	10	10
TRIAL 10 (DAY 6) - PATH B				
MEAN TIME (SECONDS)	33.34	85.42	48.59	80.54
S.D.	28.470	77.122	51.756	68.191
MEAN NO. ERRORS	5	10	8	12
S.D.	7.2	9.2	11.7	12.1
N	10	10	10	10
TRIAL 11 (DAY 7) - PATH A (PROBE)				
MEAN TIME (SECONDS)	66.84	78.34	68.22	107.81
S.D.	27.597	46.763	49.817	62.694
MEAN NO. ERRORS	15	13	18	23
S.D.	8.8	9.6	12.4	15.9
N	10	10	10	10

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

A = FORWARD ROUTE THROUGH MAZE
B = REVERSE ROUTE THROUGH MAZE

Clinical observations and survival of F1 animals during post weaning: One F1 male on day 155 at 100 mg/kg/day died, but had no clinical findings prior to the death. No other clinical findings in one dead animal or all others that survived to necropsy were attributed to the drug treatment. The F1 animal that died was internally normal, and death was attributed to the maternal drug (SCH 58235) administration. No deaths were seen in the 300-1000 mg/kg groups. However increased dried red material around the eyes/ears/nose was observed at low and high doses (total number of findings/number of pups with findings 1/1, 5/3, 0/0, 3/3 respectively). In males, hair loss in left forelimb was increased at all doses (total occurrences/number of animals 11/2, 28/4, 15/4, 12/3 respectively) and in females this was seen in both right and left forelimbs (17/4, 46/8, 63/11, 56/8 respectively) see the Table in males and females.

In scheduled necropsies, hair loss in skin was noted in 0/24, 2/22, 0/23, 1/25 F1 rats respectively (it was not specified what part of the skin in the sponsor's Table 89).

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