CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-303

PHARMACOLOGY REVIEW

DRAFT

July 18, 2001

Review and Evaluation of Pharmacology and Toxicology
Original NDA Review

NDA: 21-303

Sponsor: Shire Laboratories Inc.
Rockville, MD

Rec□d: 2/6/01

Drug: SLI 381 (Adderall [dextroamphetamine saccharate, amphetamine aspartate, dextroamphetamine sulfate, amphetamine sulfate] modified-release formulation)

Indication: ADHD

Related NDA: NDA 11-522 (Adderall)

Background:

This NDA for a modified-release formulation of Adderall relies primarily on the amphetamine literature and the limited data previously submitted to the Adderall tablet NDA (#11-522) for its preclinical information. Following negotiations between the Division and sponsor, it was agreed that the only additional animal studies required prior to approval would be a mouse micronucleus assay, the *E. coli* component of the AMES assay (to supplement a study conducted by NTP), and Segment I and II reproductive/developmental toxicology studies. These have been submitted and are reviewed below. A rat postnatal development (juvenile animal) study is to be conducted post-marketing.

APPEARS THIS WAY
ON ORIGINAL

I. GENETIC TOXICOLOGY

A) BACTERIAL MUTATION ASSAY IN *E. COLI* WP2UVRA/PKM101 (CM891) (Shire study no. V00029-SLI381-IIID; conducted by [redacted] report dated 9/1/00; GLP; Vol. 1.9)

Because an Ames test conducted by NTP did not include an *E. coli* component, it was requested that the sponsor conduct a supplemental study. In this study, Adderall (amphetamine mixed salts) was tested for mutagenicity only in *E. coli*. No increase in reverent colony numbers were seen in either of the 2 tests performed (preliminary plate incorporation test and main pre-incubation test) at concentrations of up 5000 ug (base)/plate (limit concentration according to guidelines; no cytotoxicity observed) in the presence and absence of S-9. The appropriate control responses were obtained.

B) MOUSE MICRONUCLEUS TEST (Shire study no. M00028-SLI381-IIID; conducted by [redacted] report dated 9/8/00; GLP; vol. 1.9)

In a dose range-finding test, single oral (gavage) doses of 5, 10, 12.5, and 25 mg/kg were administered to mice (2/se/group) and the animals were observed for a period of 48 hr. There was no mortality, but D-R clinical signs (including increased respiration, hyperactivity, hunched posture, piloerection, vocalization, salivation, convulsions, fasciculations, and aggression) were observed in all groups. Based on the severity of signs, 10 mg/kg was selected as the HD. There were no apparent sex differences in toxicity.

For the main study, single oral doses of 0 (vehicle), 2.5, 5, or 10 mg/kg were given to groups of male mice (14/group + 3/group for TK analysis). A group (5 males) given a dose of 12 mg/kg mitomycin C served as the positive control. (OECD guidelines allow the use of only 1 sex if it can be shown that there are no substantial differences in toxicity between sexes). Animals were killed at 24 or 48 hr after dosing, and bone marrow smears were prepared for analysis of micronucleated immature erythrocyte (PCE) frequencies and proportions of immature erythrocytes (PCE/NCE ratio). Blood samples were taken from the TK animals at 1 hr after dosing.

There was 1 death of a HD animal (4.5 hr after dosing) that was considered T-R. Clinical signs were observed at the HD only and included increased respiration, hyperactivity, piloerection, hunched posture, and fasciculations. Plasma amphetamine concentrations at 1 hr after dosing are shown in **Table IB.1**. There were no statistically significant increases ($p>0.01$) in the frequency of micronucleated PCEs at either sampling time in Adderall-treated mice (**Table IB.2**). Although there were some increases compared to the negative control values, these were not clearly D-R and were within the historical control range for the lab. Mitomycin C produced the expected increase (ss) in micronucleated PCEs but did not decrease the proportion of PCEs at 24 hr, which was the only sampling time for this group.

Table I.1 Amphetamine concentrations in mouse plasma 1 hr after dosing

Group	Animal number	Concentration (ng/ml)	Mean	sd	CV(%)
1	401	—	-	-	-
	402	—			
	403	—			
2	411	—	47.1	19.0	40.3
	412	—			
	413	—			
3	421	—	231.9	11.3	4.9
	422	—			
	423	—			
4	431	—	433.4	72.4	16.7
	432	—			
	433	—			

BLQ Below limit of quantification ——

Table I.2 Summary of micronucleus test data

Dose of Adderall mg/kg	No. of mice/ time point	Mean percentage of PCE in total of 2000 cells		Mean number of micronucleated PCE	
		24hr	48hr	24hr	48hr
0[b]	7	48	45	0.1[a]	0.1[a]
2.5	7	44	46	0.6[a]	0.3[a]
5	7	44	44	0.3[a]	0.9[a]
10	7	44	46	1.4[a]	0.1[a]
Positive Control[c]	5	50	NT	28.0[a]	NT

a In 2000 PCE

b Purified water

c Positive control is Mitomycin C at 12 mg/kg; No statistical analysis

NT Not tested

Statistically significant increases compared to solvent control (P<0.001) are indicated by a

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II) REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

A) ORAL FERTILITY AND EARLY EMBRYONIC DEVELOPMENT STUDY IN RATS (Shire Study No. R00036-SLI381-IIIB, conducted by [redacted] 1/23/01, GLP, Vol. 3.2)

1) Methods

Male and female S-D rats (22/sex/group) received oral (gavage) doses of 0 (water vehicle), 2, 6, or 20 mg free base/kg/day prior to (males 29 days, females 15 days) and during mating (up to 3 weeks of pairing), and either through Day 7 of gestation (females) or until termination during study Week 8 (males). Daily doses were divided and given 8 hr apart (i.e., 1, 3, and 10 mg/kg BID). Animals were weighed (twice weekly or daily) and observed for mortality and clinical signs (twice daily). Blood samples were collected from the orbital sinus during the week before mating for toxicokinetic analysis (2/sex/group/time at pre-dose, 1, 2, 4, 8, 9, 10, 12, 16, 20, and 24 hr after first daily dosing). Males were killed during Week 8, and their reproductive organs were weighed and examined macroscopically. Females were killed on Day 14 after mating for examination of the uterine contents.

Strain: Crl:CD(SD) IGS BR
Batch Nos.: 9E0854, 9E0851, 9E0860, 9E0857

Dose justification: Doses were based on the results of a preliminary study in males and non-pregnant females in which oral (gavage) doses of 5, 10, and 20 mg/kg were given once daily for up to 21 days. Effects consisted of D-R clinical signs and decreased food consumption and BW gain at all doses. Under the conditions of this study, 10 mg/kg was considered the MTD. 20 mg/kg was considered to exceed the MTD, based on the severity of the behavioral and BW effects. It was thought, however, that because of the rapid plasma elimination of amphetamine, 20 mg/kg/day could be tolerated when given in divided doses, and that this would also better mimic the clinical exposure pattern. In the preliminary study, Cmax values of 800 and 1458 ng/ml and AUC_{24} values of 2139 and 4909 ng.h/ml were obtained on Day 15 for males and females, respectively, at a dose of 10 mg/kg/day, once a day.

2) Results

a) F0 Effects

i) There were 4 deaths during the study. Three males (1 LD, 2HD) were killed at various times for non-treatment-related reasons (dosing, bleeding accidents). One HD female was killed on Day 2 because of self-mutilation, a typical amphetamine-induced effect in rats.

ii) Hyperactivity and sniffing of the cage were observed with dose-dependent incidences and severity at the MD and HD. All HD animals exhibited these signs during the course of the study, and they were seen throughout the treatment period. Other T-R signs, which were more prominent in males, included cage licking, salivation, piloerection, irritable behavior, and fighting (males only). Occasional cage sniffing (females only, on Day 12 only) and salivation were the only signs observed in LD animals.

iii) Body weight gain and BW were D-D decreased in MD and HD animals. In males, BW gain over the entire dosing period was 19 and 48% less than C in the MD and HD groups, respectively (**Figure IIA.1**). Mean BWs at the end of the treatment period (Day 59) were 7 and 19% below C at the MD and HD, respectively. In females, BW gain during the premating treatment period (14 days) was 27 and 73% less than in C in the MD and HD groups, respectively (**Figure IIA.2**). Mean BW prior to mating was 9% below C at the HD, but not significantly different in LD and MD group females. There were no significant differences among groups in female BW gain during gestation (**Figure IIA.3**). On Day 14 of gestation, only HD dams weighed significantly less than C (7%). Corresponding decreases in food consumption were observed during the study.

iv) There was no apparent effect of treatment on estrus cycles or on mating performance and fertility (pre-coital interval, percentage mating, conception rate, fertility index).

v) Although the absolute weights of the epididymides, prostate gland, and seminal vesicles were decreased at necropsy in HD males compared to C (6, 21, and 12%, respectively), there were no T-R differences in relative organ weights. Examination of females at necropsy did not reveal any macroscopic findings considered treatment-related.

vi) Toxicokinetics data are presented in **Table IIA.1**. The only t1/2 values that could be calculated adequately were for LD female (2.7 hr) and HD males (1.9 hr).

b) Litter Effects - C-Section Data

i) Litter parameters were unaffected by treatment (**Table IIA.2**).

Figure IIA.1 Bodyweight change - group mean values for males

Group	:	1	2	3	4
Compound	:	Control		Adderall	
Dosage (mg free base/kg/day)	:	0	2	6	20

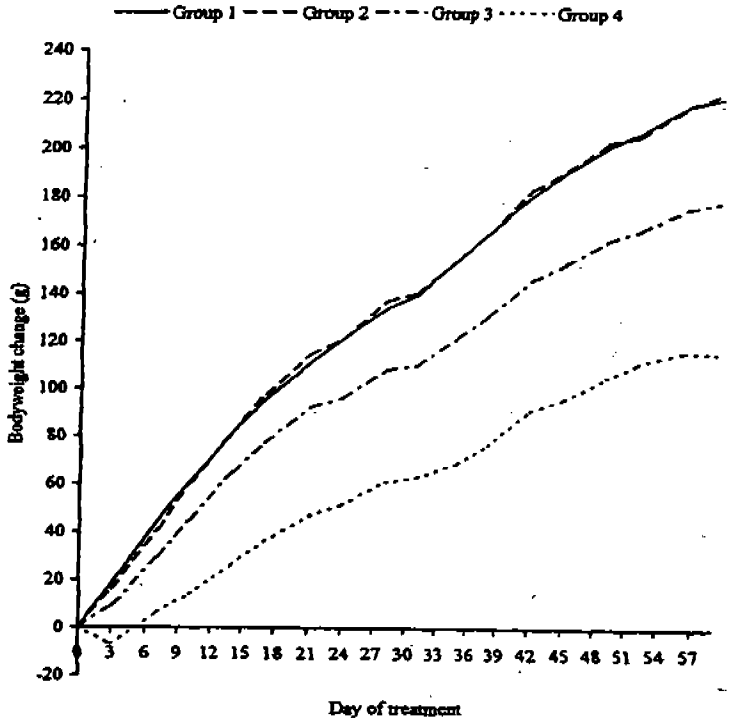

Figure IIA.2 Bodyweight change - group mean values for female prior to mating

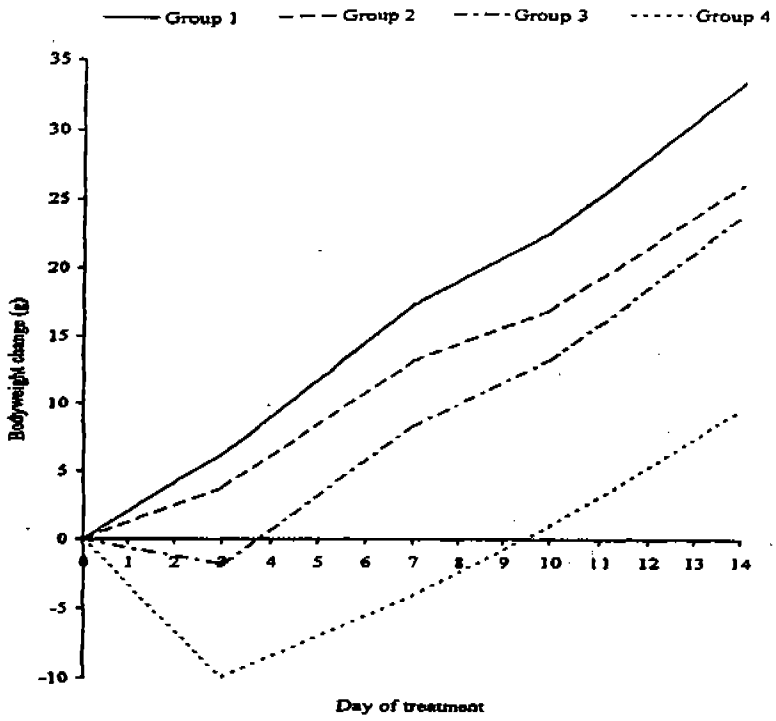

Figure IIA.3 Bodyweight change - group mean values for females during gestation

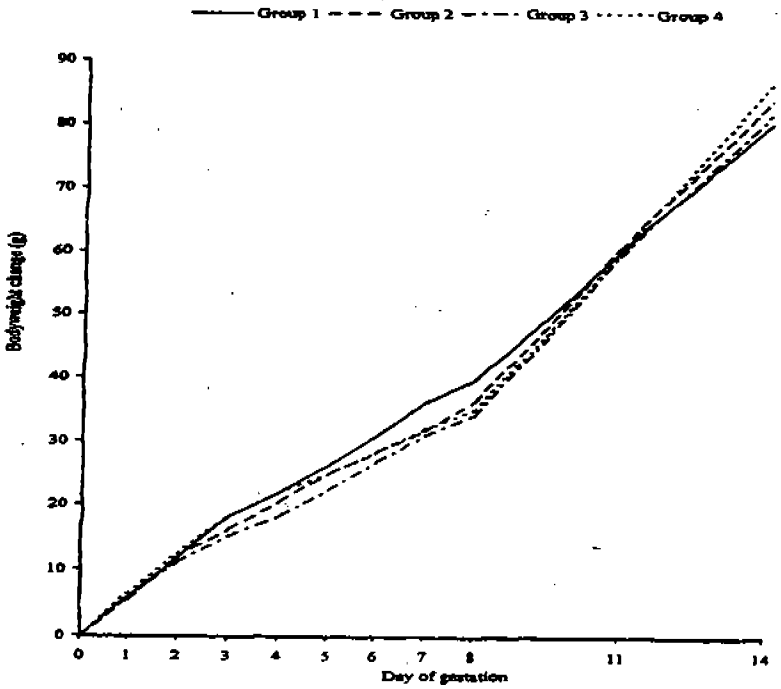

Table IIA.1 Cmax and AUC values for plasma amphetamine in rat fertility study (group means)

Dose level (mg/kg/day)	Males (*ca* three weeks of treatment) C_{max} (ng/ml) 1st dose	C_{max} (ng/ml) 2nd dose	AUC_8 (ng.h/ml)	AUC_{24} (ng.h/ml)	Females (*ca* one week of treatment) C_{max} (ng/ml) 1st dose	C_{max} (ng/ml) 2nd dose	AUC_8 (ng.h/ml)	AUC_{24} (ng.h/ml
2	39.1	46.5	100	216	81.6	81.3	236	555
6	233.9	203.4	648	1187	212.2	236.0	888	1599
20	880.4	976.1	2822	5689	1080.7	1196.9	3727	8506

Table IIA.2 Litter data for dams sacrificed on Day 14 of gestation (group means)

Group	:	1	2	3	4
Compound	:	Control		——— Adderall ———	
Dosage (mg free base/kg/day)	:	0	2	6	20

Group		Corpora Lutea	Implantations	Live Young	Resorptions Early	Resorptions Late	Resorptions Total	Implantation loss (%) Pre-	Implantation loss (%) Post-
1	Mean	16.7	15.6	14.5	1.0	0.0	1.0	6.7	7.1
	SD	1.9	2.4	2.6					
	n	22	22	22	22	22	22	22	22
2	Mean	15.9	15.5	14.5	1.0	0.0	1.0	3.1	6.7
	SD	1.8	1.8	2.8					
	n	22	22	22	22	22	22	22	22
3	Mean	16.5	16.1	15.5	0.6	0.0	0.6	3.0	3.7
	SD	1.6	1.4	1.5					
	n	22	22	22	22	22	22	22	22
4	Mean	16.8	16.2	15.1	1.1	0.0	1.1	4.9	6.6
	SD	1.7	1.9	1.8					
	n	21	21	21	21	21	21	21	21

SD Standard deviation.
n Number of pregnant females.

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B) ORAL EMBRYOFETAL DEVELOPMENT STUDY OF ADDERALL (AMPHETAMINE SALTS) IN RATS (Shire study no. R00025-SLI381-IIIC; conducted by ——— report dated 1/16/01; GLP; vol. 3.3)

1) Methods

Pregnant S-D rats (22/group) were administered 2, 6, or 20 mg free base/kg/day by oral gavage in divided doses 8 hr apart from Day 6 to Day 17 of gestation. Blood samples were taken on Day 17 at pre-dose, 1, 2, 4, 8, 9, 10, 12, 16, 20, and 24 hr after the first dose. Dams were sacrificed on Day 20 of gestation for examination of the uterine contents. Fetuses were examined for external, visceral, and skeletal abnormalities (1/2 fixed for Wilson's sectioning, 1/2 stained for skeletal examination).

Strain: Crl:CD (SD)IGS BR

Batch Nos.: 9E0854, 9E0851, 9E0860, 9E0857

Dose Justification: Doses were based on the results of a dose range-finding study in non-pregnant rats (described above).

2) Results

a) F0 Effects

i) There were 3 HD deaths, all considered T-R: 1 dam was found dead on Day 7 and 2 dams were sacrificed on Day 7 after self-mutilation of the forepaw. Because of self-mutilation in 4 additional dams and marked weight loss (mean wt loss 22 gm for GDs 6-7) at the HD, treatment at this dose was stopped and all remaining dams in this group were sacrificed on Day 9 of gestation. No additional findings were reported in these HD dams at necropsy.

ii) Clinical signs in the HD animals that died or were sacrificed included pale eyes, prostrate position, hyperactivity, sniffing of the cage, chewing of forelimbs after dosing, increased respiration, piloerection, thin build, and skin pallor. T-R clinical signs in remaining HD and MD dams consisted of increased activity, excessive head movements, head shaking, cage licking and sniffing, and salivation in most or all animals. In addition, 1 MD dam showed evidence of forelimb mutilation. Observations in the LD group were limited to 2 animals with hyperactivity and 4 with excessive head movements.

iii) Although BW loss or decreased BW gain occurred during the first few days of treatment (GDs 6-8), there was rapid tolerance development to this effect, so that for GDs 6-20 BW gain was only 7% below C at the MD. There was no significant BW effect at the LD over this period. Over GDs 6-18, BW gain was 5 and 13% below C in LD and MD dams, respectively. (See **Figure IIB.1.**)

iv) Toxicokinetic data are presented in **Table IIB.1**. No blood samples were taken from the HD group because of early termination. The t1/2 values were determined to be 3.0 and 2.3 hr at 2 and 6 mg/kg/day, respectively.

b) Litter Effects

i) There were no apparent effects on litter parameters (**Table IIB.2**) or on placental, fetal, and total litter weights.

ii) Fetal structural changes were classified according to severity and incidence as major abnormalities, minor abnormalities, or variants. There were no apparent effects of treatment on incidences of major abnormalities (**Table IIB.3**) or minor visceral abnormalities. A possible effect on cranial ossification was seen, however. Incomplete ossification of the cranial centers (considered a minor skeletal abnormality) was found in 11, 14, 18% of fetuses in the C, LD, and MD groups, respectively (historical control range: 3-10%). Although litter incidences were not clearly increased (10/22, 8/22, and 12/22 in C, LD, and MD groups, respectively), the number of litters containing 3 or more affected fetuses was increased in both treated groups compared to C (**Table IIB.4**). No additional T-R differences in the incidences of minor skeletal abnormalities

or variants were found.

Figure IIB.1 Bodyweight change - group mean values for females during gestation

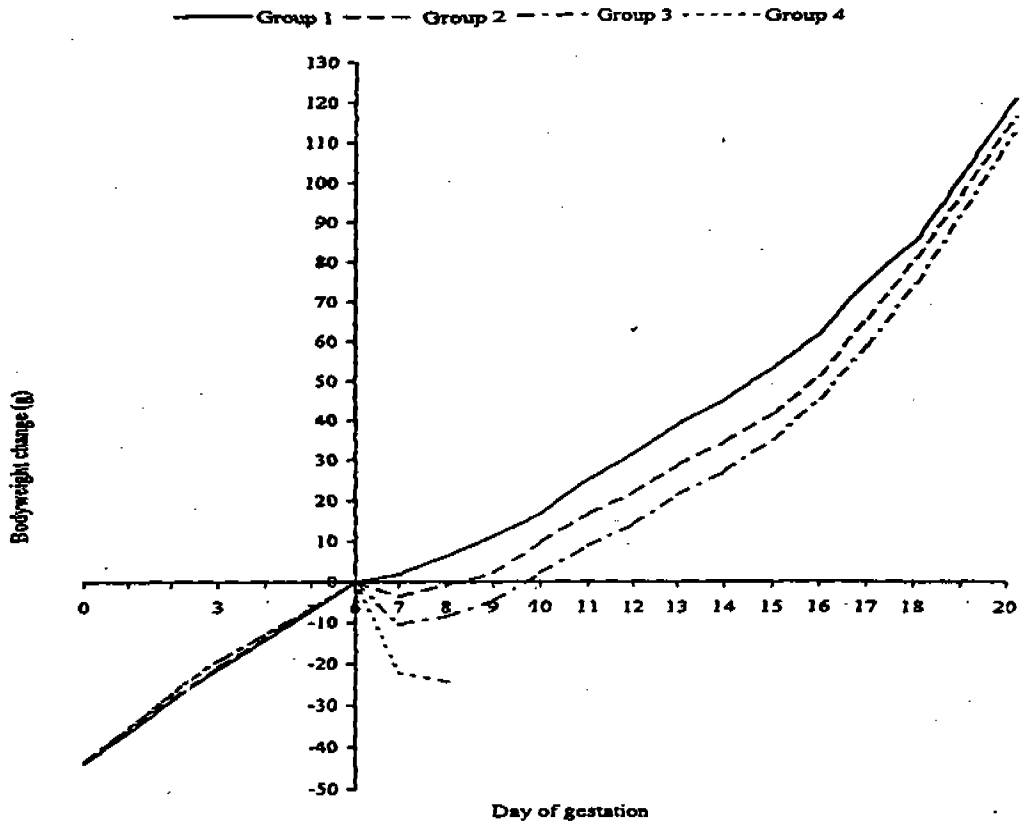


Table IIB.1 Cmax and AUC values for plasma amphetamine in rat embryofetal development study

Dose level (mg/kg/day)	C_{max} (ng/ml) 1st dose	C_{max} (ng/ml) 2nd dose	AUC_0 (ng.h/ml)	AUC_{24} (ng.h/ml)
2	80.3	93.2	221	455
6	248.6	233.8	700	1566

Table IIB.2 Litter data in rat embryofetal development study - group mean values

Group	:	1	2	3	4
Compound	:	Control	---------- Adderall ----------		
Dosage (mg free base/kg/day)	:	0	2	6	20

Group		Corpora Lutea	Implantations	Resorptions			Live young			Sex ratio	Implantation loss (%)	
				Early	Late	Total	Male	Female	Total	(% M)	Pre-	Post-
1	Mean	15.8	15.1	0.7	0.0	0.8	7.5	6.9	14.4	52.1	4.2	5.1
	SD	2.1	1.7				2.0	2.2	1.7			
	n	22	22	22	22	22	22	22	22	22	22	22
2	Mean	15.7	15.1	1.0	0.0	1.0	7.0	7.1	14.1	49.6	5.1	6.7
	SD	1.7	1.4				1.7	2.0	1.7			
	n	22	22	22	22	22	22	22	22	22	22	22
3	Mean	16.1	15.3	0.5	0.0	0.5	7.5	7.2	14.8	51.1	5.9	3.3
	SD	2.3	1.4				2.2	2.3	1.5			
	n	22	22	22	22	22	22	22	22	22	22	22
4	Mean		14.3									
	SD		1.4									
	n		18									

SD Standard deviation.
n Number of females.

Table IIB.3 Group incidences of major abnormalities in rat embryofetal development study

	Fetuses			Litters		
Group	1	2	3	1	2	3
Dose (Adderall mg/kg/day)	0	2	6	0	2	6
Number examined	316	310	325	22	22	22
Number affected	1	1	2	1	1	2
Dilated aortic arch; aorticopulmonary fistula	1	-	-	1	-	-
Medially thickened/kinked ribs, marked: irregularly ossified ribs	-	1	-	-	1	-
Markedly reduced right testis and seminal vesicle	-	-	1	-	-	1
Atrial and ventricular septal defects: complete situs inversus	-	-	1	-	-	1

Table IIB.4 Fetuses/litter with incomplete cranial ossification in rat embryofetal development study

		Litters		
Group		1	2	3
Dose (Adderall mg/kg/day)		0	2	6
Number examined		22	22	22
Number of fetuses with incomplete ossification				
Cranial centres	0	12	14	10
	1	4	2	3
	2	5	2	3
	3	1	3	4
	4	0	0	2
	>4	0	1	0

C) ORAL EMBRYOFETAL DEVELOPMENT STUDY IN RABBITS (Shire study no. L00037-SLI381-IIIC; conducted by ——————— report dated 1/22/01; GLP; vol. 3.3)

1) Methods

Presumed pregnant rabbits (22/group) were administered 2, 6, or 16 mg free base/kg/day by oral gavage in divided doses 8 hr apart from Day 6 to Day 19 of gestation. Because of concern about excessive toxicity at the original HD of 20 mg/kg/day, the study was run in 2 phases: 6 females per group were treated in Phase 1, and the remaining animals were treated in Phase II. Due to the severity of post-dosing signs in the 6 animals receiving 20 mg/kg/day in Phase I, it was decided that this dose exceeded the MTD, and for Phase II another full group (n=22) was started at the revised HD of 16 mg/kg/day. Blood samples were taken on Day 19 at pre-dose, 1, 2, 4, 7.5, 9, 10, 12, 15.5, 20, and 24 hr after the first dose. Dams were sacrificed on GD 29 for examination of the uterine contents, and fetuses were examined for external, visceral, and skeletal abnormalities (fresh dissection method followed by staining for skeletal examinations; 1/3 of fetuses decapitated and heads fixed for serial sectioning).

Strain: New Zealand White

Batch Nos. (amphetamine salts): 9E0854, 9E0851, 9E0860, 9E0857

Dose Justification: Doses were based on the results of a dose range-finding study in non-pregnant rabbits (3/dose) in which doses of 10, 20, and 50 mg/kg/day were given once daily for up to 14 days. All 3 HD animals were sacrificed on the first day of dosing due to severe clinical signs (abnormal gait, hyperactivity, dilated pupils, convulsion, prone posture, and gasping). One MD doe was also sacrificed on Day 2 with similar observations. Necropsy exams revealed pink frothy material in the trachea, lung congestion, and a firm left ventricle of the heart in these animals that were sacrificed ("for reasons of animal welfare"). Clinical signs in LD and remaining MD animals consisted of D-R increases in dilated pupils, increased respiration, and hyperactivity. There were no BW effects at any dose. It was concluded that 10 mg/kg was the MTD, but since amphetamine is rapidly eliminated, it was decided that this could be tolerated BID.

2) Results

a) F0 Effects

i) The only T-R death was that of 1 HD (20 mg/kg/day) doe that was sacrificed on GD 12 after showing pronounced behavioral changes (listed below). There were no deaths in the remaining groups (16 mg/kg/day or lower) except for 1 C doe that died as a result of a dosing accident.

ii) Findings at 20 mg/kg/day included what were described as severe behavioral changes (dilated pupils, increased respiration, hyperactivity, repetitive chewing and cage floor scraping, abnormal gait/incoordination, prostrate posture, increased body temperature) in 1 doe that was sacrificed on GD 12. Clinical signs observed in the remaining animals dosed with 20 mg/kg/day as well as in those receiving the new HD of 16 mg/kg/day consisted primarily of hyperactivity, repetitive movements, dilated pupils, and increased respiration (D-R incidence and severity). Dilated pupils were also seen in 1 MD doe. No clinical signs were observed at the LD.

iii) There were no effects of treatment on BW gain (**Figure IIC.1**) or food consumption.

iv) Toxicokinetic data are presented in **Table IIC.1**.

b) Litter Effects

i) There were no clear effects on litter parameters, although a slight increase in post-implantation loss was seen at 16 mg/kg/day (**Table IIC.2**). Placental, fetal, and total litter weights were comparable among groups with the full complement of litters. Decreased fetal weights were seen at 20 mg/kg/day, however (9% below C). This was thought to be a possible consequence of the greater mean litter size in this group.

ii) Fetal structural changes were classified according to severity and incidence as major abnormalities, minor abnormalities, or variants. There were no apparent effects of treatment on incidences of major abnormalities (**Table IIC.3**) or minor visceral abnormalities.

iii) The proportion of fetuses with 13 ribs (considered a skeletal variant by this lab) was increased somewhat in both HD litters compared to C (**Table IIC.4**). This effect was considered (in study report) of questionable relation to treatment. No additional group differences in incidences of minor skeletal abnormalities/variants were found.

Figure IIC.1 Bodyweight change - group mean values for females during gestation

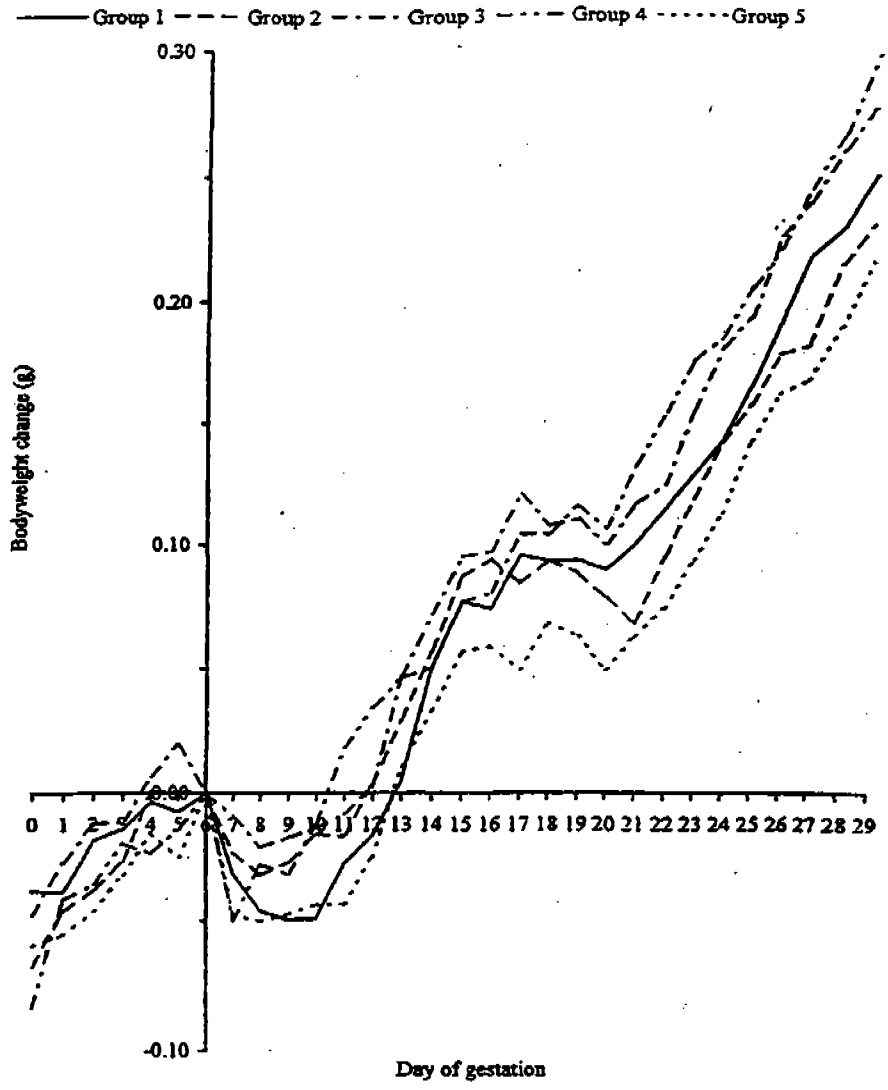


Table IIC.1 Cmax and AUC values for plasma amphetamine in rabbit embryofetal development study

Dose level (mg/kg/day)	C_{max} (ng/ml) 1st dose	2nd dose	$AUC_{7.5}$ (ng.h/ml)	AUC_{24} (ng.h/ml)
2	22.7	36.2	33.95	89.85
6	61.9	158.5	121.69	376.77
16	258.9	359.9	588.23	1464.42

Table IIC.2 Litter data in rabbit embryofetal development study - group mean values

Group	:	1	2	3	5	4
Compound	:	Control		---------- Adderall ----------		
Dosage (mg free base/kg/day)	:	0	2	6	16	20

Group		Corpora Lutea	Implantations	Resorptions			Live young			Sex ratio (% M)	Implantation loss (%)	
				Early	Late	Total	Male	Female	Total		Pre-	Post-
1	Mean	9.8	8.5	0.4	0.9	1.2	3.6	3.7	7.3	52.2	13.1	12.0
	SD	1.9	2.6				1.6	1.9	2.0			
	n	20	20	20	20	20	20	20	20	20	20	20
2	Mean	9.9	8.2	0.3	0.9	1.2	3.2	3.8	7.0	44.7	15.8	13.7
	SD	1.6	1.9				1.8	1.4	1.8			
	n	21	21	21	21	21	21	21	21	21	21	21
3	Mean	9.4	8.2	0.4	0.6	1.0	3.6	3.6	7.2	51.0	13.1	11.2
	SD	1.4	2.0				1.6	2.0	1.7			
	n	21	21	21	21	21	21	21	21	21	21	21
5	Mean	9.5	8.3	0.4	1.1	1.5	3.1	3.6	6.8	46.5	12.6	17.2
	SD	1.2	2.4				1.9	2.0	2.5			
	n	22	22	22	22	22	22	22	22	22	22	22
4	Mean	10.2	9.8	0.2	0.8	1.0	5.0	3.8	8.8	56.3	4.6	9.7
	SD	1.9	0.8				1.9	1.5	0.8			
	n	5	5	5	5	5	5	5	5	5	5	5

Group		Placental weight	Litter weight	Fetal weights		
				Males	Females	Overall
1	Mean	6.4	344.3	48.7	47.1	48.0
	SD	1.1	88.0	4.6	4.9	4.7
	n	20	20	20	19	20
2	Mean	5.9	321.2	46.6	46.7	46.4
	SD	0.8	78.5	4.7	4.4	4.1
	n	21	21	21	21	21
3	Mean	6.0	339.5	47.1	47.6	47.5
	SD	0.7	83.1	5.7	5.1	5.2
	n	21	21	20	21	21
5	Mean	6.4	315.6	47.5	47.2	48.1
	SD	1.3	107.0	6.0	5.3	5.9
	n	22	22	20	20	22
4	Mean	5.9	386.1	43.3	43.3	43.6
	SD	0.6	70.9	6.8	4.9	5.0
	n	5	5	5	5	5

Table IIC.3 Group incidences of major abnormalities in rabbit embryofetal development study

	Fetuses					Litters				
Group	1	2	3	5	4	1	2	3	5	4
Dose (Adderall mg/kg/day)	0	2	6	16	20	0	2	6	16	20
Number examined	146	147	151	149	44	20	21	21	22	5
Number affected	0	10	4	1	1	0	4	4	1	1
Cebocephaly with fused nasals overlying frontals, single medial naris, palatine irregularities, absent upper incisor sockets, incisors and tongue, agnathia, incomplete inferior vena cava with persistent right posterior cardinal vein	-	1	-	-	-	-	1	-	-	-
Cebocephaly, single medial naris, palatine irregularities, brachygnathia with fused mandibles, small lower incisor sockets, absent upper and lower incisors, cervical vertebral irregularities	-	1	-	-	-	-	1	-	-	-
Cebocephaly, medially displaced nares, absent upper incisors with fused small incisor sockets	-	1	-	-	-	-	1	-	-	-
Cebocephaly, absent upper incisors	-	-	-	-	1	-	-	-	-	1
Medial cleft lip and palate, nasal furrow, misshapen bilateral parietal, frontal, nasal, maxilla, premaxilla, partially fused parietal to interparietal, displaced upper incisors, dorsoventral distortion sternum	-	-	-	1	-	-	-	-	1	-
Cervical vertebral irregularities	-	1	-	-	-	-	1	-	-	-
Systemic/pulmonary artery abnormality/ventricular septal defect	-	2	3	-	-	-	2	3	-	-
Incomplete inferior vena cava with persistent left posterior cardinal vein	-	-	1	-	-	-	-	1	-	-
Lumbar spina bifida occulta, vertebral irregularities, protrusion occipital region	-	1	-	-	-	-	1	-	-	-
Lumbar scoliosis	-	2	-	-	-	-	1	-	-	-
Conjoint twins, cranioschisis, open right eye, fused nasals, premaxillae and upper incisor sockets, single upper incisor, ventricular septal defect, incomplete inferior vena cava with persistent posterior cardinal vein	-	1	-	-	-	-	1	-	-	-

Table IIC.4 Incidences of minor skeletal abnormalities/variants in rabbit embryofetal development study

		Fetuses					Litters				
Group		**1**	**2**	**3**	**5**	**4**	**1**	**2**	**3**	**5**	**4**
Dose (Adderall mg/kg/day)		**0**	**2**	**6**	**16**	**20**	**0**	**2**	**6**	**16**	**20**
Number examined		146	137	147	148	43	20	21	21	22	5
Number intact		98	86	98	102	28	20	20	21	22	5
Cranial	sutural bone	1	2	1	1	-	1	1	1	1	-
	fissure/additional suture	1	2	3	2	-	1	2	3	2	-
	bridge of ossification	-	1	-	1	-	-	1	-	1	-
	bent cornua of hyoid	1	1	6	1	-	1	1	6	1	-
Vertebral element	abnormality										
	cervical	2	1	-	-	-	2	1	-	-	-
	thoracic	1	1	-	1	-	1	1	-	1	-
	additional ossified centre	-	2	1	-	-	-	2	1	-	-
	scoliosis minimal	1	-	-	1	-	1	-	-	1	-
Ribs	medially thickened	1	1	1	1	-	1	1	1	1	-
	branched/fused/absent	-	1	1	1	-	-	1	1	1	-
	misshapen/offset/misaligned	2	-	-	-	-	2	-	-	-	-
Sternebrae	bridge of ossification/fused	1	1	1	1	-	1	1	1	1	-
	additional centre	-	1	1	-	-	-	1	1	-	-
	offset/bipartite ossification	-	1	1	-	-	-	1	1	-	-
	wide/fragmented	1	1	-	-	-	1	1	-	-	-
Costal cartilage	offset	1	1	-	1	-	1	1	-	1	-
	additional	1	1	-	1	-	1	1	-	1	-
	fused/branched/interrupted	1	2	-	3	1	1	2	-	3	1
Limb	small claw	-	-	-	1	-	-	-	-	1	-
Total affected by one or more of the above		**8**	**14**	**15**	**12**	**1**	**8**	**10**	**10**	**9**	**1**
Rib and vertebral configuration											
Cervical rib		2	2	3	2	-	2	2	1	2	-
Short 1st rib		-	-	-	2	-	-	-	-	2	-
Short/absent 12th rib		-	-	2	-	-	-	-	1	-	-
Number with 12/13 or 13/13 ribs		84	86	91	102	30	20	20	20	21	4

III. SUMMARY AND EVALUATION:

The two genetic toxicology studies performed by the sponsor (Ames *E. coli* component, mouse micronucleus test) were negative, but reports in the literature indicate that amphetamine may have genotoxic potential. In an Ames test conducted by [redacted], d,l- amphetamine (100, 333, 1000, 3333, and 10000 ug/plate) was negative in tester strains TA100, TA155, and TA1537 in the presence and absence S9 and was negative in tester strain TA98 in the absence of S9, but produced what was considered an equivocal response in TA98 in the presence of S9 (positive, equivocal, and weakly positive in 4, 10, and 20% rat S9, respectively). In other tests conducted by [redacted] (sister chromatid exchange and chromosomal aberrations in CHO cells), d,l-amphetamine was negative with and without S9. In another study reported in the literature, d,l-amphetamine tested positive in a mouse micronucleus assay (Tariq et al., Mutation Res. 190:153-57,1987). When mice were given 2 oral doses of 0, 6.25, 12.5, 18.75, or 25.0 mg/kg each, significant increases in micronucleated PCEs were observed at doses of 12.5 mg/kg or greater. These results are in contrast to those obtained by the sponsor, but the conditions of the 2 studies were sufficiently different to allow for both being valid.

In carcinogenicity studies of d,l-amphetamine conducted by [redacted] which were considered adequate for the NDA, rats (F344/N; 50/sex/grp) and mice (B6C3F1; 50/sex/grp) were fed diets containing 0, 20, or 100 ppm for 2 years ([redacted] summarized in **Table III.1**). These doses were based on the results of 13-week dose range-finding studies at doses of up to 750 ppm in rats and 2000 ppm in mice. In the 2-year studies, there were no significant differences in survival among groups of rats or mice. Hyperactivity was observed in all treated groups of rats and mice. Final BWs were 92 and 86% of C for LD and HD male rats, 89 and 70% of C for LD and HD female rats, 85 and 72% of C for male mice, and 81 and 66% of C for LD and HD female mice. Food consumption was similar among C and exposed groups with the exception of HD female rats (84% of C) and HD male mice, for which hyperactivity resulted in scattering of feed and overestimation of consumption. The average amount of dl-amphetamine consumed per day was estimated to be, respectively, 1 and 5 mg/kg for LD and HD rats, 4 and 30 mg/kg for LD and HD male mice, and 3 and 19 mg/kg for LD and HD female mice. There were no D-R increases in neoplasms in rats or mice receiving amphetamine. In fact, administration of amphetamine was associated with decreases in the incidence of total neoplasms and in the incidences of certain site-specific tumors, including pheochromocytomas of the adrenal gland in male rats, fibroadenomas of the mammary gland in female rats, adenomas of the anterior pituitary in male and female rats and in female mice, and adenomas or carcinomas (combined) of the liver in male and female mice. It was concluded that, under the conditions of these studies, there was no evidence of carcinogenic activity of dl-amphetamine in rats or mice. No plasma levels were obtained, but based on estimated daily administered doses, these studies provide little or no safety margin relative to human doses. The HD in rats (5 mg/kg = 30 mg/m2) is equivalent to the maximum dose in children (30 mg/25 kgX25 = 30 mg/m2), while the HDs in male (30 mg/kg = 90 mg/m2) and female (19 mg/kg = 57 mg/m2) mice are only 3- and 2-fold the highest clinical dose, respectively.

No clear evidence of reproductive or developmental toxicity was identified in studies conducted by the sponsor (rat fertility, rat and rabbit embryofetal development). Although the dose-selection process was faulty (dose range-finding studies were conducted in non-pregnant animals under dosing conditions different than those used in the definitive studies), doses that were at least minimally maternally (paternally) toxic were evaluated in all 3 studies. It is likely that a dose higher than 6 mg/kg/day could have been tolerated by dams in the rat embryofetal development study, however, and the suggested effect on cranial ossification seen in treated litters in this study could have been better assessed had an appropriate HD been used. But there was no other indication of an effect on morphological development in rats, and the only possible effects seen in rabbits were slight increases in resorptions and in the fetal incidence of a skeletal variation (13 ribs) in litters exposed to the highest dose. These essentially negative findings agree with the limited data reported in the literature for amphetamine (d- and d,l-) in rats and rabbits (reviewed by Schardein in: Chemically Induced Birth Defects, 2nd edition, Dekker, New York, 1993, pp. 223-225). Teratogenic effects have been reported with d-amphetamine in mice, but only at higher, maternally lethal doses (50-100 mg/kg "injected"). The safety margins provided by the current studies are minimal to non-existent, however. At the highest dose examined in the rat fertility study (20 mg/kg/day), mean total exposures in males and females (AUC_{0-24}: 5689 and 8506 ng.hr/ml, respectively; non-stereoselective analysis) were about 3 and 5 times, respectively, those measured in children receiving the maximum clinical dose of 30 mg (AUC_{0-24}: 1800 ng.hr/ml total amphetamine; 1364 ng.hr/ml d-amphetamine,

443 ng.hr/ml l-amphetamine). At the highest doses adequately tested in the rat and rabbit embryofetal development studies (6 and 16 mg/kg/day, respectively), mean total exposures (AUC_{0-24}: 1566 and 1464 ng.hr/ml) were less than those seen clinically. If peak concentrations are compared, the values in the rat fertility study (M: 976 ng/ml; F: 1197 ng/ml) were about 10-fold those in children receiving a dose of 30 mg (Cmax: 117 ng/ml total amphetamine: 89 ng/ml d-amphetamine, 28 ng/ml l-amphetamine), while the peak levels in the rat and rabbit embryofetal development studies (249 and 360 ng/ml, respectively) were about 2 and 3-fold, respectively, those measured clinically. Because it is not known which PK parameter is more important for the teratogenic response, and because there is no information on concentrations of amphetamine enantiomers in the plasma of animals given Adderall salts, it may be preferable to use administered dose (mg/m2) when making animal to human comparisons.

While there is little evidence that amphetamine is teratogenic, at least not at exposures similar to those seen clinically, a number of studies indicate that prenatal or early postnatal exposure to drugs in this class, at relatively low doses (generally 0.5-5 mg/kg, sc or ip), can have long-term neurochemical and behavioral consequences for rodent offspring (reviewed by sponsor, Vol. 1.9). The commonly reported behavioral effects include learning and memory deficits, altered locomotor activity, and changes in sexual function (possibly neuroendocrine-related). Neurochemical studies indicate that early amphetamine exposure can produce long-lasting changes in various indices of monoaminergic synaptic function (receptor density, neurotransmitter content, etc.). Amphetamines can also have long-term effects on monoaminergic systems in the adult brain, including nerve terminal degeneration, but the neurotoxic effects produced in developing animals appear to be qualitatively different than those seen in adults (Pu and Vorhees, Dev Brain Res 72:325-328,1993). The sensitive period for the developmental neurotoxicity of the amphetamines is thought to be during synaptogenesis (late prenatal and early postnatal periods of development), when the substrate for drug interaction (i.e., monoaminergic nerve terminals) is expressed in the CNS. This has been systematically examined with methamphetamine, which produced the most pronounced learning deficits in rats when exposure occurred during a specific period of postnatal development (PND 11-20; Vorhees et al., Psychopharmacology 114:392-401,1994). Behavioral effects have also been reported in rats after earlier prenatal methamphetamine exposure, however (Acuff-Smith et al., Neurotoxicol Teratol 18:199-215,1996). The sponsor has acknowledged the developmental neurotoxicity of amphetamine. In their pharmacology summary, they conclude, "Prenatal exposure to amphetamine is associated with a variety of responses in offspring that included increases in conditioned avoidance, exploratory activity, sexual behavior, and decreases in 5-HT content in the medial hypothalamus and in the number of spontaneously active cells in the locus coeruleus nucleus." Regarding the adult neurotoxicity of amphetamine, they state, "Repeated administration of high concentrations of amphetamine produced striatal, neostriatum, and frontal cortex dopamine nerve fiber degeneration." Similar statements should appear in the label.

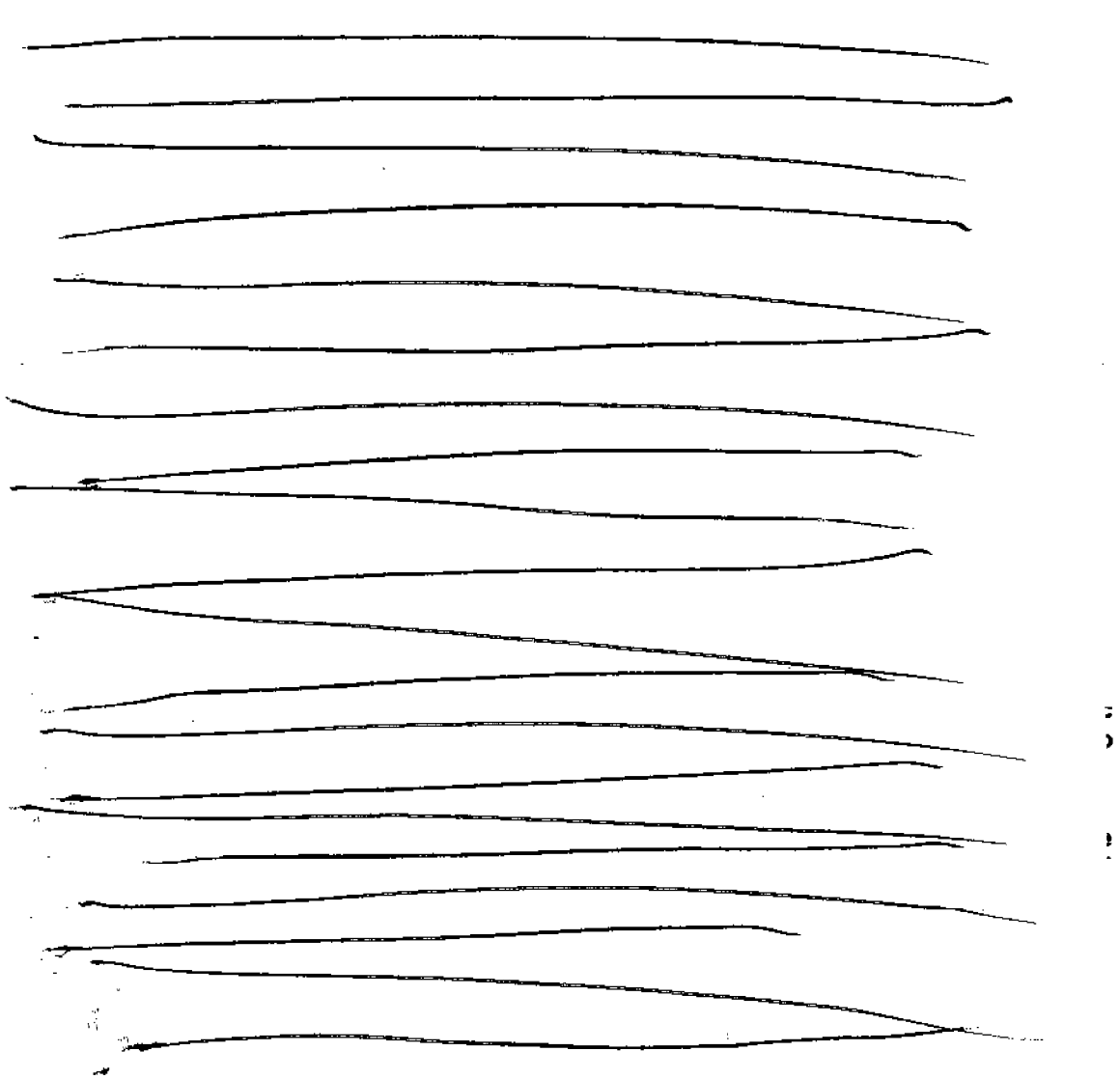
Table III.1

Summary of the 2-Year Feed and Genetic Toxicology Studies of *dl*-Amphetamine Sulfate

Variable	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses	0, 20, or 100 ppm *dl*-amphetamine sulfate	0, 20, or 100 ppm *dl*-amphetamine sulfate	0, 20, or 100 ppm *dl*-amphetamine sulfate	0, 20, or 100 ppm *dl*-amphetamine sulfate
Body weights	Dosed groups markedly lower than controls	Dosed groups markedly lower than controls	Dosed groups markedly lower than controls	Dosed groups markedly lower than controls
2-Year survival rates	30/50, 31/50, 33/50	33/50, 42/50, 37/50	48/50, 48/50, 49/50	35/50, 36/50, 44/50
Nonneoplastic effects	None	None	None	None
Neoplasms decreasing	Adrenal pheochromocytomas: 23/49, 15/44, 6/50 Anterior pituitary gland adenomas: 15/49, 15/48, 7/49	Mammary gland fibroadenomas: 21/50, 11/50, 2/50 Anterior pituitary gland adenomas: 31/50, 24/48, 19/50 Endometrial stromal polyps: 10/50, 6/50, 3/50	Harderian gland adenomas: 4/50, 2/50, 0/50 Lung adenomas or carcinomas (combined): 8/50, 3/50, 4/50 Liver adenomas or carcinomas (combined): 14/50, 12/50, 2/50)	Anterior pituitary gland adenomas: 12/49, 6/49, 1/46 Harderian gland adenomas: 5/50, 2/50, 0/47 Lung adenomas or carcinomas (combined): 8/50, 6/50, 1/47 Liver adenomas or carcinomas (combined): 5/50, 1/50, 1/47
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA100, TA1535, and TA1537. Equivocal with S9 in strain TA98; negative without S9			
Sister chromatid exchanges				
Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Chromosomal aberrations				
Chinese hamster ovary cells *in vitro*:	Negative with and without S9			

APPEARS THIS WAY
ON ORIGINAL

RECOMMENDED LABELING:



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IV. RECOMMENDATIONS

The NDA is approvable with respect to the pharmacology/toxicology portion. Recommendations concerning the proposed labeling are made in Summary and Evaluation section of the review.

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cc:
NDA (21-303)
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J.E. Fisher, Ph.D.