

7) Oral Pre-and Postnatal Toxicity Study of HWA 486 (A77 1486A) Including Maternal Function in Wistar Rats. Document 13395	22
8) Toxicokinetics of A77 1726 Following Administration of HWA 486 in an Additional Study Designed to Evaluate the Toxicokinetics in an Oral Rabbit Embryotoxicity Study (Toxicology Study N ^o 97.0124). Report N ^o 16702	25
Mutagenicity Studies	
1) A77 1486 (HWA 486) Ames Test (Salmonella/Mammalian-Microsome Mutagenicity Test - Standard Plate Test) and Prival Modification (Salmonella/Mammalian Microsome Mutagenicity Test - Preincubation Test). Document 8407	26
2) A Mutagenicity Screening of HWA 486 in Bacteria (Ames Test). Document 8475	27
3) Evaluation of A77 1486 in the Unscheduled DNA Synthesis Assay in Mammalian Cells in Vitro. Document 10326	27
4) A77 1486 Detection of Gene Mutations in Somatic Cells in Culture HGPRT Test with V79 Cells. Document 8780	28
5) A77 1486 (HWA 486) Micronucleus Test in Male and Female NMRI Mice after Oral Administration. Document 10091	29
6) Evaluation of A77 1486 (HWA 486) in the In Vivo Cytogenetic Test in Bone Marrow Cells of the Chinese Hamster - Chromosome Analysis. Document 10470	30
7) 4-Trifluoromethylaniline Study of the Mutagenic Potential in Strains of Salmonella Typhimurium (Ames Test) and Escherichia Coli. Document 12797	31
8) 4-Trifluoromethylaniline Ames Test (Salmonella/Mammalian Microsome Mutagenicity Test - Standard Plate Test) and Prival Modification (Salmonella-Mammalian Microsome Mutagenicity Test - Preincubation Test). Document 8508	32
9) 4-Trifluoromethylaniline Detection of Gene Mutations in Somatic Cells in Culture HGPRT - Test with V79 Cells. Document 13344	33
10) 4-Trifluoromethylaniline Micronucleus Test and Female NMRI Mice After Intraperitoneal Administration. Document 13086	34
11) 4-Trifluoromethylaniline Chromosome Aberrations In Vivo Cytogenetic Test in Bone Marrow Cells of the Chinese Hamster. Document 14161	34

12) 4-Trifluoromethylaniline Chromosome Aberrations In Vitro in V79 Chinese Hamster Cells. Document 13555

35

ABBREVIATIONS USED IN THIS REVIEW

D = day(s)	G = group(s)	DR = dose related	W = week(s)
	↑ = increase(s)	↓ = decrease(s)	
* = p<0.05	** = p<0.01	*** = p<0.001	# = p<0.0001

Oral Fertility Study of HWA 486 (A77 1486) in Wistar Rats (Effect on Fertility, Pregnancy and Postnatal Development) Segment I.

Document 10463.

NDA 20-905, Volume 1.39-1.40, p

Report N^o: 92.0763; Study N^o: 90.1036

Compound: HWA 486 (A 77 1486), Batch N^o U 003

Formulation: Suspension in starch mucilage at concentrations of 0.06, 0.25, and 0.80 g drug/L.

Feed/Water: Altromine® 1310, both ad libitum.

Route: Oral, by stomach tube, once daily at 5 mL/kg body weight.

Dosages: Group 1: 5.00 mL starch mucilage/kg (control), equivalent to 100 mg starch/kg

Group 2: 0.40 mg HWA 486/kg

Group 3: 1.25 mg HWA 486/kg

Group 4: 4.00 mg HWA 486/kg

Control Treatment: Potato starch mucilage (20 g/L in distilled H₂O)

Strain: Wistar [Hoe:WISKf(SPF71)]: mean body wt- ♂ 124±9 g, age at start 30-35 days
mean body wt- ♀ 193±7 g., age at start 70-75 days

Number: 32 sex/group in P generation, 1-11/sex/group in F₁ generation

Study Site: Pharma Development/Central Toxicology, Hoechst AG, Frankfurt/Main

Date: December 6, 1990 - October 6, 1992

GLP/QAU Statements: Both present and signed.

The purpose of this study was to evaluate the fertility, pregnancy, and postnatal development of Wistar rats administered HWA 486 (A 77 1486) daily for seventy days prior to mating and through mating for males and from 14 days prior to mating, during mating, pregnancy, and through lactation until weaning in females.

Doses were selected from a preliminary embryotoxicity study using 0, 5, 10, 15, 20, and 30 mg/kg. At 30 mg/kg, fetal deaths occurred. Slight embryotoxicity occurred at 20 mg/kg. At ≥ 10 mg/kg, malformations of the head, rump, vertebral column, ribs, and limbs occurred. It was stated that no complications occurred in the dams or conceptuses at 5 mg/kg, and the 10 and 15 mg/kg doses "did not adversely affect the health of the dams." The 20 mg/kg dose was "slightly embryotoxic and the 30 mg/kg dose led to fetal death." In addition, studies in rats showed

mortality at 2 mg/kg. Based on these data, the above doses (0.4, 1.25, 4.0 mg/kg) were selected for the Segment I study. The low dose (0.4 mg/kg) is the maximum daily therapeutic dose intended in humans.

In the present study, ♂ were treated daily for at least 70 days prior to mating and during mating. The ♀ were treated without interruption from 14 days prior to the start of mating, during mating, during pregnancy, and during the lactation period. HWA 486 was prepared fresh daily. Animal behavior and general physical condition were evaluated daily. Body weight and food consumption were determined weekly, except for P♀ during mating and after parturition. Food consumption was not monitored in ♂ from the beginning of mating onward. Twenty ♀ from each group were killed day 21, the dams C sectioned, and the uterus and fetuses examined. Sires of the P and of the F₁ generation were killed and dissected either after the ♀ had mated or after they had littered. Their organs were examined macroscopically and the testes weights. All remaining 12 ♀ were allowed to litter and rear their pups. These pups were evaluated during lactation, tested for hearing, vision, and for righting reflex. Behavior and memory tests were also evaluated. Motor activity, coordination, and sense of balance were also checked in each pup.

Pups selected for the F₁ generation were examined for physical development and for functional and behavioral disorders. When mature for breeding, their fertility, parturitional, and lactational capabilities were evaluated. There were 11/sex F₁ dams in the control and 0.4 mg/kg group; however, due to mortality in the P generation, there was only 1/sex F₁ in the 1.25 mg/kg group and none in the 4.0 mg/kg F₁ group to continue the study.

RESULTS

P Generation:

- mortality: • 1 ♂ G2 killed D13 of pretreatment due to malnutrition from an additional incisor which prevented the animal from eating - this animal was replaced-
- 1 ♂ G2 and 1 ♂ G4 killed due to intubation error following mating-
 - 1 ♂ G4 killed D23 during pretreatment period - not available for mating-
- clinical signs: • 1 ♀ G3 vaginal bleeding D14-D17 after second mating - animal was killed D17-
- 1 G4 ♂ red discolored urine at times during pretreatment and mating-
 - local alopecia in all groups - increased urine excretion-
- body weight: slight retardation * (2%-4%) in ♂ G4 during treatment-
- ! G2 lactation D0 (*12%) - food consumption not changed from control values-

mating results:

Nº of Days From Start of Mating Until Sperm Detected in Vaginal Smear

DAYS	CONTROL N = 32	0.4 MG/KG N = 32	1.25 MG/KG N = 32	4.00 MG/KG N = 32
1-5	21+1 ^a	21	23	19
6-10	3+1 ^a	3	4+1 ^a	8
11-15	2	2	2	4
16-20	2	2	-	-
21-25	1	1	1	1
26-30	-	1	1	-
31-35	1	1	-	-
36-40	-	1	-	-
41-45	-	-	-	-
46-50	-	1	-	-
51-55	-	-	-	-
56-60	-	1	-	-

^a Pregnant without sperm detected.

- estrous was present within 1 to 5 days from the start of vaginal smears-
- pregnancy occurred after three matings in most animals-

Nº of Matings Required By Females For Pregnancy to Occur

MATINGS	CONTROL N=32	0.40 MG/KG N=32	1.25 MG/KG N=32	4.0 MG/KG N=32
1	17+2 ¹	16	16	14
2	5	7	7+1 ¹	11
3	3	4	2	4
4	-	2	2	1
5	2	1	-	-
6	-	1	-	-
10	-	1	-	-
Non-pregnant	3 (2 ²)	-	4 ²	2 (1 ²)

¹ Pregnant without sperm detected.

² Nº of these allocated to cesarean section group.

- 1 ♂G1, 2 ♂G2, 1 ♂G3 were infertile [testicular atrophy (↓ weight) in 1G2 and 1G3]-
- ♂ with spermatozoa/no pregnancy- 3G1, 4G3, and 1G4-
- ♂ without spermatozoa/no pregnancy- 2G1, and 3G2-
- 1 ♀G3 killed prematurely, subsequent to vaginal bleeding - found dead embryofetal primordia in the uterus-

- cesarean section data:

**Results During Gestation and Cesarean Section
(Vol. 1.39, pp. 5-9996 and 5-9997)**

	Group 1 0.00 mg/kg	Group 2 0.40 mg/kg	Group 3 1.25 mg/kg	Group 4 4.00 mg/kg
N ^o ♀ with spermatozoa	20	20	20	20
Pregnancies	18	20	16	19
♀ Abortions	0	1	0	0
♀ With Premature Delivery	0	0	0	0
♀ At Term With Intrauterine Deaths Only	0	0	0	1
♀ At Term With Live Fetuses:				
Corpora lutea total (mean ± SD)	18 (100%) 304 (16.9 ± 2.1)	19 (95%) 315 (16.6 ± 2.0)	16 (100%) 262 (16.4 ± 2.2)	18 (95%) 305 (16.9 ± 2.3)
Implantations (mean ± SD)	283 (15.7 ± 2.0)	283 (14.9 ± 3.4)	236 (14.8 ± 2.1)	256 (14.2 ± 2.8)
Pre-implantation Loss - Mean %	6.68	10.80	9.85	15.85
Post-implantation Loss - Mean %	5.14	4.57	7.94	12.69
Early intrauterine deaths total (mean ± SD)	15 (0.83 ± 0.79)	9 (0.47 ± 0.51)	19 (1.19 ± 1.17)	30 (1.67 ± 1.68)*
Late intrauterine deaths total (mean ± SD)	0 (0.00 ± 0.00)	0 (0.00 ± 0.00)	0 (0.00 ± 0.00)	1 (0.06 ± 0.24)
Total intrauterine deaths (mean ± SD)	15 (0.83 ± 0.79)	9 (0.47 ± 0.51)	19 (1.19 ± 1.17)	31 (1.72 ± 1.74)*
Live fetuses total (mean ± SD)	268 (14.9 ± 1.9)	274 (14.4 ± 3.6)	217 (13.6 ± 2.2)	225 (12.5 ± 3.3)*

* p<0.05

THE NUMBER OF FETUSES AND LITTERS EXAMINED

	GROUP 1 0.00 mg/kg	GROUP 2 0.40 mg/kg	GROUP 3 1.25 mg/kg	GROUP 4 4.00 mg/kg
External/Visceral: N ^o of Fetuses Examined	139	140	111	116
N ^o of Litters Examined	18	19	16	18
Skeletal Defects: N ^o of Fetuses Examined	139	140	111	116
N ^o of Litters Examined	18	19	16	18

**RESULTS IN LIVE FETUSES AT CESAREAN SECTION
(VOL. 1.39, P. 5-9997)**

	GROUP 1 0.00 mg/kg	GROUP 2 0.40 mg/kg	GROUP 3 1.25 mg/kg	GROUP 4 4.00 mg/kg
Total N ^o of Fetuses	268	274	217	225*
% of implants mean	94.86	95.43	92.06	87.31
% Males	44.78	45.26	51.15	50.67
Body Weight g Mean (SD)	3.2 (0.3)	3.2 (0.3)	3.1 (0.3)	3.0 (0.4)*
Crown/Rump Length Mean mm (SD)	34.5 (1.9)	34.8 (2.1)	34.4 (2.1)	33.7 (2.1)
Placental Weight Mean g (SD)	0.47 (0.09)	0.45 (0.07)	0.44 (0.07)	0.43 (0.06)

* p<0.05

- duration of pregnancy (G1-G4)- 22.6, 22.8, 22.9, 22.5 days- no complications-
- 1G1 fetus with lateral transposition of viscera of abdomen + fused lung lobes + undeveloped lung lobe-

- 1 G2 fetus with aplasia of right eye lense - 1 with blood near right kidney-
- no malformations in any G3-
- 1 G4 fetus with asymmetry of nose tip + aplasia of right eye lense - 1 G4 fetus with gastroschisis - 1 G4 fetus with blood blister on dorsum of left hind leg foot-
- 1 fetus in each dose group with hematoma in one hepatic lobe-
- individual fetuses in all groups with distention of one or both renal pelvis-
- 1-3 fetuses in all groups with distention of one or both ureters-
- slight retardation in skeletal ossification in G4 (vertebra, sternebra, 5th metacarpal bones, and feet phalanges)-
- offspring survival rate (%) at 21 days- G1 (99.4%), G2 (98.7%), G3 (8.4%), G4 (0.0%)-

SIGNIFICANT (*) INTERGROUP SKELETAL DEFECTS IN FETUSES
(Vol. 1.39, 5-10005)

	GROUP 1 0.00 mg/kg	GROUP 2 0.40 mg/kg	GROUP 3 1.25 mg/kg	GROUP 4 4.00 mg/kg
Caudal Vert Centra: (retardation) Ossification of <2 Vertebral Centers:				
Fetuses (%)	29 (20.9)	28 (20.0)	35* (31.5)	44* (37.9)
Litters (%)	11 (61.1)	9 (47.4)	11 (68.8)	14 (77.8)
Anlage of a 14th Thor. Vert. With Analogous 14th Rib: (variation)				
Fetuses (%)	0	2 (1.4)	(0.9)	10* (8.6)
Litters (%)	0	2 (10.5)	1 (6.3)	6* (33.3)
Sternebra: Not or Weakly Ossifies: (retardation)				
Fetuses (%)	45 (32.4)	50 (35.7)	41 (36.9)	65* (56.0)
Litters (%)	14 (77.8)	14 (73.7)	15 (93.8)	17 (94.4)
Forepaw: Non-Ossified Metacarpal 5 (retardation)				
Fetuses (%)	51 (36.7)	63 (45.0)	55* (49.5)	75* (64.7)
Litters (%)	15 (83.3)	17 (89.5)	14 (87.5)	16 (88.9)
Forepaw - Phalanx: 3 of 1st to 5th Toes Non-Ossified (retardation)				
Fetuses (%)	0	3 (2.1)	0	5* (4.3)
Litters (%)	0	1 (5.3)	0	2 (11.1)
Hind paw - Phalanx 3 of 1st to 5th Toes Non-Ossified (retardation)				
Fetuses (%)	15 (10.8)	18 (12.9)	12 (10.8)	51* (44.0)
Litters (%)	7 (38.9)	5 (26.3)	7 (43.8)	14* (77.8)

* p <0.05 one sided

● lactation period:

The body weight of dams during lactation (D0 to D21) was slightly lower in G3 and G4. On the other hand, the body weight of G3 offspring began to differ significantly from G1 by D7, and by D21 the survival rate was 8.4%, with only one litter surviving the lactation period. More extreme body weight changes occurred in G4. These pups were only slightly lighter than G1 pups at birth, but body weight became markedly reduced after only four days. By D14 all pups in G4 were dead or had to be killed moribund. None survived the lactation period.

The following table (Vol. 1.40, p. 5-10128) indicates the results at birth, during weaning, and to sacrifice.

	Control	0.40 mg/kg	1.25 mg/kg	4.00 mg/kg
Experimental ♀ with sperm/pregnant	12/11	12/12	12/12	12/11
Pregnant ♀ - that died	0	0	0	0
- with implants only	0	0	0	0
- with dead offspring only	0	0	0	0
- which delivered normally	11	11	11	11
Body weight gain (g) in dams (Days 0 - 21)	117	109	122	110
Implantations per dam	14.1	14.0	15.8	15.3
Live offspring per dam	13.4	13.2	14.8	13.5
Dead offspring per dam	0.0	0.0	0.2	0.0
Supernumerary implantation sites per dam	0.7	0.8	0.8	1.7
Body wt (g) of offspring/dam at - birth (mean)	5.67 ± 0.34	5.62 ± 0.51	5.53 ± 0.39	5.29 ± 0.62
- 4 days after birth (mean)	9.1 ± 0.7	9.4 ± 2.0	8.5 ± 0.6	6.9 ± 0.8*
- 7 days after birth (mean)	13.1 ± 1.2	12.9 ± 2.3	11.1 ± 1.0	5.8 ± 0.5
- 14 days after birth (mean)	23.4 ± 2.3	23.9 ± 4.0	14.6 ± 3.9	-
- 21 days after birth (mean)	36.2 ± 4.2	35.9 ± 7.5	27.1 ± 0.0	-
Sex of live offspring ♂ (♀) %	48 (52)	52 (48)	52 (48)	51 (49)
Survival rate (%) at 21 days	99.4	98.7 #	8.4 #	0.0
Unreared litters	0	0	10	11
Weanlings with external perceptible abnormalities	0	0	11	11
Weanlings with internal abnormalities	0	0	0	0

* significantly different from control ($p < 0.05$)

statistical evaluation not possible

The general behavior and physical condition of G2 pups were normal during the lactation period. Abnormal posture and movement of the limbs, mostly the front limbs, were observed in eight G3 and nine G4 litters. In addition, a paleness of the feet and ankles was present in these two groups. Tails of these two groups were also wavy, with kinks also present in wavy tails in two litters of G4. Tail bones were indicated as not being deformed; it was assumed that wavy tails were related to the joint changes. Suckling was reduced in nine G3 and ten G4 litters. Corneal opacity occurred in one or both eyes of five pups in one G3 litter during the third week. The physical development and results from the function tests (hearing, vision, righting reflex, motor activity, coordination, balance) of surviving treated pups of G2 and G3 (only one litter) did not differ from control pups, but those pups in G3 with opaque corneas had no pupil response to light. Functional tests could not be done in G4, as all pups had died.

Eleven pups/sex in G1 and G2 and only 1/sex from G3 were available to continue the

study in the F₁ generation. Fertility and impairment of pregnancy and parturition in these F₁ animals were not altered, nor was the post natal development of F₂ animals impaired. The percent of males to females was slightly increased from G1 (46/54) to G2 (49/51) to G3 (54/46) in the F₂ generation.

The results of oral administration of HWA 486 at 0.40 mg/kg (2.4 mg/m²), 1.25 mg/kg (7.5 mg/m²) and 4.0 mg/kg (25 mg/m²) did not impair mating or reduce the number of pregnant rats. Spermatogenesis, however, was not evaluated. These doses were not toxic to the parents, but the 1.25 and 4.0 mg/kg doses were toxic to the offspring, causing weight loss, mortality, wavy/kinky tails, abnormal posture and limb movement, reduced suckling, and corneal opacity. There was a dose-related increase in intrauterine embryofetal mortality. The low dose had a slight mean percent increase in the pre-implantation loss. Surviving F₁ offspring did not show an impairment in fertility, pregnancy, parturition, or in postnatal development. At histopathology, no toxic effects to internal organs were related to the drug in any of the generations.

**Dose-Finding Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Wistar Rats
(Segment II)**

Document 10477.

NDA 20-905, Vol. 1.41, pp. 5-10604 to 5-10873

Document N^o: 010477, Report N^o 91.0068

Compound: HWA 486 (A 77 1486 A), Batch N^o U 003

Formulation: Suspensions in potato starch mucilage

Route: Oral, by stomach tube once daily from D7 - D16 of pregnancy at 5 mL/kg.

Food/Water: Ad libitum

Strain: Wistar, 65-70 days old, 190±11 g body weight

Number: 21 ♀ in G1, 3 ♀ in G2-5, 10 ♀ in G6.

Dosages: Group 1: 0, 5 mL vehicle/kg

Group 4: 15 mg/kg

Group 2: 5 mg/kg

Group 5: 20 mg/kg

Group 3: 10 mg/kg

Group 6: 30 mg/kg

Vehicle: Starch mucilage (20 g potato starch/L distilled water)

Study Site: Pharma Research, Hoechst AG, Frankfurt/Main

Date: January 25, 1991

GLP/QAU Statements: Not present.

The purpose of the study was to evaluate the embryo toxicity of HWA 486. The 21 rats in the control group were from another study that was performed at the same time as the dose-finding study. Dose-finding studies were indicated as being conducted in compliance with GLPs, and are usually described in the final reports of main studies to justify dosages tested. GLP and QAU statements were not required. The drug was examined in increasing doses, using three pregnant animals per dose. The high dose was increased to 10 animals in order to verify the results from the other groups.

Drug concentrations were prepared fresh daily. Animal behavior and general physical condition were evaluated daily. Food consumption and body weight were recorded weekly. All animals were killed Day 21 after mating and the fetuses delivered by caesarean section. Uteri and fetuses were examined. Dams were dissected, the organs examined macroscopically, and heart, liver, kidney, and spleen weights recorded. Mean values and standard deviations were calculated for many parameters.

RESULTS

- behavior and general physical condition were not widely impaired-
 - piloerection 1 dam G6 - alopecia 1 dam each G1, G2, and G5-
- food consumption not affected in G1-G5 dams - slight ↓ in G6-
- body weight of dams: DR ↓ G2 to G6 from D14-D17 and D17-D21-
G6 ↓ 11% D14-17 and ↓ 13% D17-D21-
- 2G6 dams with empty implantation sites - 3G6 dams with dead embryonic primordia-
- 2/10 dams aborted in G6 - 3/10 intrauterine deaths G6-
- post-implantation loss G1 (7.1%) vs G6 (31.4%)-
- dam liver weight was reduced 16% in G6 - indicated as being within normal range-
- litter size ↓ G6 due to 50% fetal deaths - (3 total resorptions and 2 abortions)-
- G5 and G6 fetuses ↓ body weight (15.6% G5, 18.8% G6) - ↓ body length-
- observed fetal malformations: head (hydrocephalus, microphthalmia, anophthalmia, absence of eye lens, fusions and dysplasia of head bones, fissures/fusions of jaw bone), rump, vertebral column (fusion of the exoccipital bones and 1st cervical vertebra), rib fusion/deformations, and limbs- other malformations include schisis and diaphragmatic hernia, anlage of only six cervical vertebrae, 14th thoracic vertebra with an analogous rib, 7th lumbar vertebra, irregular ossification of sternum, additional ribs, or fragments of ribs - other findings may be found in the following summary of major morphological findings-

SUMMARY OF MAJOR MORPHOLOGICAL FINDINGS IN LIVE FETUSES

(From Vol.1.41, pp. 5-10720 to 5-10731)

DOSE mg/kg	5	10	15	20	30
EXTERNAL/VISCERAL DEFECTS AT AUTOPSY					
N° OF FETUSES EXAMINED/N° OF LITTERS EXAMINED	22/3	19/3	19/3	19/3	26/5
EXTERNAL					
Retarded fetuses	0/0	0/0	3/2	3/1	10/3
HEAD					
Encephalocle in region of frontal bone	0/0	0/0	0/0	0/0	1/1
Facial part of skull asymmetrical	0/0	0/0	0/0	0/0	7/2
Transparent blister in region of frontal or parietal bone	0/0	0/0	0/0	0/0	2/1
Hematocyst in region of frontal/parietal bone	0/0	0/0	0/0	0/0	1/1

DOSE mg/kg	5	10	15	20	30
JAWS					
Agnathia inferior or brachygnathia superior and/or inferior	0/0	0/0	0/0	0/0	6/3
ORAL CAVITY					
Cheilognathoschisis superior - uni- or bilateral	0/0	0/0	0/0	0/0	5/1
EYES					
Anophthalmia - uni- or bilateral	0/0	0/0	0/0	3/2	16/5
Microphthalmia - uni- or bilateral	0/0	1/1	0/0	5/3	5/2
Aplasia lentis - uni- or bilateral	0/0	1/1	0/0	4/3	2/1
BRAIN					
Hydrocephalus internus	0/0	4/1	1/1	5/2	16/5
THORACIC CAVITY					
Thoracoschisis with protrusion of heart	0/0	0/0	0/0	0/0	2/2
LUNGS / DIAPHRAGM					
Diaphragmatic hernia - left and left half of lungs displaced - craniad	0/0	0/0	0/0	0/0	1/1
FORELIMB					
Grown to elbow into arm fold, not freely mobile	0/0	0/0	0/0	0/0	9/4
SKELETAL DEFECTS					
SKULL					
Orbit reduced in size - uni- or bilateral	0/0	1/1	0/0	7/3	21/5
Nasal and/or basioccipital bone - dysplasia	0/0	0/0	0/0	0/0	3/1
Exoccipital fused with 1 st cervical vertebra - uni- or bilateral	0/0	3/1	2/1	0/0	12/5
Frontal and/or parietal bone - only marginally performed	0/0	0/0	0/0	0/0	4/2
Opening in parietals - circular and large - right	0/0	0/0	0/0	0/0	1/1
Epactal bone between both parts of nasal/frontal bone	0/0	0/0	0/0	0/0	1/1
Cranium protruding in region of frontal/parietal bone	0/0	0/0	0/0	0/0	6/2
INCISORS AND JAWS					
Incisors, alveoli and rami of mandible fused	0/0	0/0	0/0	0/0	3/2
CERVICAL VERTIBRAL ARCHES					
Aplasia, partial aplasia, dysplasia, fragmented, fused uni- or bilateral	0/0	5/1	2/1	5/3	19/5
THORACIC VERTEBRAE / RIB					
Aplasia of 3 rd thoracic vert. arch & 3 rd rib - left, dysplasia of 4 th rib & weakly ossification of 13 th thoracic vert. arch - left, aplasia of 3 rd and dysplasia of 4 th thoracic vert. centra	0/0	0/0	0/0	1/1	0/0
THORACIC VERTEBRAL ARCH					

DOSE mg/kg	5	10	15	20	30
Aplasia and dysplasia or fused	0/0	0/0	0/0	0/0	4/2
THORACIC VERT. CENTRA					
Fragmented, aplasia, longitudinally displaced or dislocated	0/0	1/1	1/1	3/2	9/4
EXTRA VERTEBRAE / EXTRA RIB					
Additional thoracic vert. with analogous rib - unilateral	0/0	0/0	0/0	0/0	3/2
FUSED RIBS					
At media, distal, or proximal part - unilateral	0/0	0/0	0/0	0/0	3/2
EXTERNAL/VISCERAL DEFECTS OBTAINED AT BODY CROSS-SECTION					
Nº OF FETUSES EXAMINED/Nº OF LITTERS EXAMINED	21/3	16/3	19/3	18/3	22/5
HEAD					
Cranium protruding in region of frontal/parietal bone	0/0	0/0	0/0	0/0	3/1
EYES					
Anophthalmia - uni- or bilateral	0/0	1/1	0/0	6/2	14/5
Microphthalmia - uni- or bilateral	0/0	1/1	1/1	1/1	7/3
BRAIN					
Hydrocephalus internus	0/0	3/1	0/0	9/3	18/5
DIAPHRAGM / LUNGS					
Diaphragmatic hernia - left and left half of lungs displaced - craniad	0/0	0/0	0/0	0/0	2/2
Diaphragmatic hernia - right	0/0	0/0	0/0	0/0	1/1

- no major findings in G1 (control)-
- no major findings in G2 (5 mg/kg)-

The administration of HWA 486 was tolerated up to 15 mg/kg by the dams. Food consumption was slightly reduced (23%) D14-D17 of gestation in G6, and body weight was reduced 11%-13% over D14-D21. Group 5 body weight was reduced, but was stated as being within normal limits. Only at 5 mg/kg was this compound non-toxic to fetuses; however, the teratogenic effects at 10, 15, 20 and 30 mg/kg were significant, occurring in increasing number of litters and fetuses.

Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Wistar Rats (Effect on Morphological Development) ICH 4.1.3**Document 13220.****NDA 20-905, Vol. 1.41, pp. 5-10874 to 5-11056.**Study N^o: 94.0110, Report N^o: 95.0246,Compound: HWA 486 (A 77 1486 A), Batch N^o L 029-1

Formulation: Suspension prepared each day in starch mucilage.

Route: Oral, by stomach tube once/day at 5 mL/kg from 7th - 19th day of pregnancy.

Food/Water: Ad libitum.

Strain: Wistar, approximately 65-75 day old, 189.3 ± 11.3 g mean body weight

Number: 22 mated ♀/group

Dosages: Group 1 0 (5 mL starch mucilage/kg)

Group 2 1 mg/kg

Group 3 15 mg/kg

Vehicle: Starch mucilage (20 g potato starch/L distilled water)

Study Site: Pharma Development, Corporate Toxicology, Hoechst AG, Frankfurt/Main

Date: February 25, 1994 - July 6, 1995

GLP/QAU Statements: Both present and signed.

This study was conducted to identify the maximum dose of HWA 486 (A 77 1486 A) that can be tolerated by dams and their conceptuses when given orally to pregnant rats from pregnancy Day 7 to pregnancy Day 19, the sensitive period of organogenesis. On the 21st day after mating the rats were killed and the fetuses delivered by caesarean section. The examinations included autopsy and skeleton analysis in about 50% and body-cross sections after Wilson in about 50%.

RESULTS

- no impairment of dam behavior or general physical condition-
- food consumption significantly ↓ from control in 15 mg/kg dams D7-14 (10.8%) and D14-D21 (6.3%)-
- body weight of dams ↓ 7.1%* D14, 10.3% D17, 11.7% D21 at 15 mg/kg-
- at autopsy of dams:
 - 1G2 with light-brown cortex at cranial and caudal pole of kidney-
 - 1G1 and 3G2 with dilated renal pelvis (one or both)-

Gestational and Caesarean Section Results
(From Vol. 1.41, p. 5-10912 and p. 5-10913)

	GROUP 1 0 mg/kg	GROUP 2 1 mg/kg	GROUP 3 15 mg/kg
PREGNANCIES TOTAL	20	20	20
FEMALES AT TERM WITH LIVE FETUSES TOTAL	20	20	20
CORPORA LUTEA TOTAL MEAN (S.D.)	272 13.6 (1.7)	283 14.2 (1.7)	273 13.7 (4.0)
IMPLANTATIONS TOTAL MEAN (S.D.)	251 12.6 (2.7)	267 13.4 (1.5)	262 13.1 (3.4)
PRE-IMPLANTATION LOSS % MEAN	7.7	5.25	5.87
POST-IMPLANTATION LOSS % MEAN	8.68	5.23	29.20
% OF IMPLANTATIONS	8.68	4.89	25.90*
LIVE FETUSES TOTAL MEAN (S.D.)	231 11.6 (3.1)	253 12.7 (1.6)	182 ^b 9.1 (3.4)

*Significantly higher than control

^bSignificantly less than control

- corpora lutea and implants similar to control-
- mean loss of early implantations- G1 (8.68%), G2 (4.89%), G3 (25.9%)*
- % of implantations- (mean post-implantation loss % minus % mean of implantations) G1 (0.00%), G2 (0.33%), G3 (3.30%)-
- live fetuses reduced in 15 mg/kg due to embryofetal deaths-
- conceptuses undergoing resorption and dead fetuses ↑ at 15 mg/kg (*)

RESULTS IN LIVE FETUSES AT CAESAREAN SECTION
(From Vol. 1.41, p. 5-10914)

	GROUP 1 0 mg/kg	GROUP 2 1 mg/kg	GROUP 3 15 mg/kg
NUMBER OF FETUSES TOTAL MEAN (S.D.)	231 11.6 (3.1)	253 12.7 (1.6)	182* 9.1 (3.4)
% OF IMPLANTATIONS MEAN	91.32	94.77	70.80*
CROWN/RUMP LENGTH MM MEAN (S.D.)	35.2 (1.3)	34.9 (1.3)	32.3 (1.4)*
PLACENTAL WEIGHT, GRAMS MEAN (S.D.)	0.47 (0.05)	0.44 (0.04)	0.36 (0.04)*
FETAL WEIGHT, GRAMS MEAN (S.D.)	3.4 (0.3)	3.4 (0.2)	2.8* (0.2)
N° OF LITTERS	20	20	19

*Significantly less than control

- sexes were evenly balanced-
- 92 fetuses from the 19 litters in G3 exhibited the following malformations-
 - brain (internal hydrocephalus) - eyes (anophthalmia/microphthalmia, oval-shaped lens), cervical vertebral column (aplasia, partial aplasia, dysplasia, fusions)-
 - many combined malformations-
- other G3 malformations - edema in subcutis, encephalocele near frontal/parietal bone, asymmetry of viscerocranium, brachygnathia inferior, partial fusion of both rami of

- mandible, diaphragmatic hernia and scoliosis of thoracic vertebral column-
- delayed skeletal ossification at 15 mg/kg - other findings are indicated in the following summary table-

SUMMARY OF MAJOR FINDINGS IN LIVE FETUSES
(From Vol. 1.41, pp. 5-10916 to 5-10925)

DOSE (mg/kg)	0	1	15
EXTERNAL/VISCERAL DEFECTS OBTAINED AT AUTOPSY			
Nº OF FETUSES EXAMINED / Nº OF LITTERS EXAMINED	120/20	131/20	96/20
EXTERNAL			
Retarded fetuses	1/1	0/0	12/10
BODY			
Edematous fetus	0/0	0/0	1/1
HEAD			
Cranium protruding in region of frontal or parietal bone	0/0	0/0	3/2
Facial part of the skull - asymmetric	0/0	0/0	1/1
JAW			
Brachygnathia inferior	0/0	0/0	1/1
EYE			
Anophthalmia or microphthalmia - uni- or bilateral	0/0	0/0	29/13
BRAIN			
Hydrocephalus internus	0/0	0/0	28/15
DIAPHRAGM / LUNG / LIVER			
Diaphragmatic hernia - left lung - left half of lungs displaced - craniad lung - lobus sinister - reduced in size, liver - lobus sinister and lobus accessorius displaced to thoracic cavity, liver - lobus dexter, lobus dexter accessorius and lobus caudatus - fused	0/0	0/0	1/1
EXTERNAL / VISCERAL AND SKELETAL DEFECTS			
Retarded fetus; brain - skull, forelimb, forepaw multiple defects	0/0	0/0	1/1
EYE			
Anophthalmia - right, microphthalmia - left, orbit reduced in size - bilateral; skull/cervical vert. arch - dysplasia 2nd - right, fused 3rd and 4th - ventrad - left; brachygnathia superior; jaw - mandible fuses - partly; cheilognathoplatoschisis - left; facial part of the skull - asymmetrical; encephalocele in region of parietal bone, nasal bone - dysplasia, frontal, parietal and interparietal bone - only marginally preformed	0/0	0/0	1/1
SKELETAL DEFECTS			
Nº OF FETUSES EXAMINED / Nº OF LITTERS EXAMINED			
SKULL			
Orbit - reduced in size - uni- or bilateral	0/0	0/0	25/13
Exoccipital bone - dysplasia - uni- or bilateral	0/0	0/0	9/4

DOSE (mg/kg)	0	1	15
INCISOR / JAW			
Mandible fuses - partly	0/0	0/0	2/2
SKULL / CERVICAL VERT. ARCH			
Os exoccipita bone fuses with 1st cervical vertebra; anlage of only 4, 5, or 6 cervical vertebral arches only dorsal part developed, aplasia, partial aplasia, dysplasia, fused	0/0	0/0	39/18
VERTEBRAL COLUMN / RIB			
Scoliosis in region of thoracic vertebrae - sinistrad thoracic vert. Centra - dislocated, fragmented longitudinally displaced 4th, 7th, 11th, 12th fused vert. Arches - 3rd, 4th cervical arch - right fused vert. Arches - 5th, 6th thoracic arch - right rib - vestigial 7th - right fused ribs - 10th, 11th proximal part - right	0/0	0/0	4/4
EXTERNAL / VISCERA DEFECTS OBTAINED AT BODY CROSS-SECTION EXAMINATION			
Nº OF FETUSES EXAMINED / Nº OF LITTERS EXAMINED	111/20	122/20	86/20
EXTERNAL			
Retarded fetus	1/1	0/0	11/9
HEAD			
Encephalocele in region of frontal and parietal bone	0/0	0/0	1/1
JAW			
Brachygnathia inferior	0/0	0/0	1/1
EYE			
Anophthalmia or microphthalmia - uni- or bilateral	0/0	0/0	23/10
Lens dysplastic - oval shaped - left	0/0	0/0	1/1
BRAIN			
Hydrocephalus internus	0/0	0/0	31/14

At 1 mg/kg, no effects were seen on pregnancy or intrauterine development. Only minor irregularities in the skeleton or internal organs occurred at this dose and can be considered to be spontaneous. Results at 15 mg/kg, however, clearly revealed the teratogenic potential of this drug, with 51% of all fetuses in the 19 litters developing malformations. The predominant malformations were concentrated in the brain (33% internal hydrocephalus), eyes (23% anophthalmia or microphthalmia), and 23% malformations in the cervical vertebral column. This dose was also slightly toxic to the dams.

A77 1486 (HWA 486) and A77 1726 Study of the Teratogenic Properties in Rat Embryo Limb Bud Cells.

Document 11505

NDA 20-905, Vol. 1.41, pp. 5-11057 to 5-11102

This study looked at rat embryo limb bud cells to determine the teratogenic potential of HWA 486 (A77 1486) and its metabolite A77 1726 in inhibiting cell proliferation. Thirteen-day old

embryo cells from ten mated female Sprague Dawley rats were cultured 5 days in the presence of 0.25, 0.5, 1, 2, 4, 8, 16, 31, 62, 125, and 250 µg/mL dissolved in the culture medium (HAM-F12, 10% FCS) + 0.5% DMSO. Cell proliferation was assessed by measuring the intensity of stain taken up by the cells. Roussel-UCLAF (France) conducted the study in January 1992.

From Vol.1.41, p. 5-11069

MEAN CONCENTRATIONS µg/mL					
COMPOUND	IP50	ID50	Cytostatic Concentration	ICT50	ICC50
HWA 486	1.0	1.2	2 - 16	39.0	55.5
A77 1727	0.5	0.6	1 - 8	50.0	49.3

IP50 = inhibition proliferation 50%
 ID50 = inhibition differentiation 50%
 ICT50 = cell death of 50% of cultured cells
 ICC50 = cell death of 50% of chondroblasts

The results of the study indicated that HWA 486 and metabolite A77 1726 inhibited both cell proliferation and cell differentiation. Cytostatic and cytotoxicity were also noted. The parent drug was half as active in proliferation and differentiation as the metabolite (A77 1727). Concentrations that were active were stated as being of the same order as 5 FU tested under the same conditions in this study.

Dose-Finding Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Himalayan Rabbits (Segment II).

Document 10476

NDA Vol. 1.42, pp. 5-11103 through 5-11364

Report N^o: 91.0067, Document N^o: 10476

Compound: HWA 486 (A77 1486A)

Formulation: Suspension prepared fresh daily in starch mucilage.

Food/Water: Both ad libitum.

Route: Oral, once daily from D6 - D18 of pregnancy.

Strain: Himalayan [(Hoe:HIMK(SPFWiga)], 7-10 months old, 2.661±0.109 kg mean body wt.

Number of Animals: G1 (15), G2 (2), G3 (5), G4 (2), G5 (10), G6 (2), G7 (2), G8 (11).

Dosages:

G1: control 5 mL starch mucilage/kg	G5: 16 mg/kg
G2: 5 mg/kg	G6: 20 mg/kg
G3: 10 mg/kg	G7: 25 mg/kg
G4: 15 mg/kg	G8: 30 mg/kg

Vehicle: Starch mucilage (20 g potato starch/L distilled water)

Study Site: Hoechst AG, Pharma Research, Frankfurt/Main

Date: February 25, 1991

GLP/QAU Statements: Not required.

This was a dose finding study to determine the dosages for the main teratology study. Ascending doses were evaluated in 2 to 11 animals. Pregnant rabbits from another study being

At 0, 5, and 10 mg/kg, no effect on intrauterine development of conceptuses was noted, and their body weight, development, and length of live C-section fetuses were normal. Embryo primordia were present at ≥ 15 mg/kg. In addition, the number of females with abortions clearly indicated embryonic toxicity. Live fetuses obtained after cesarean sectioning from these dams, however, showed normal development, but the ability to survive was greatly decreased.

Malformations were not observed in fetuses from dams treated with ≤ 20 mg/kg. The four fetuses from the one surviving 30 mg/kg dam exhibited uni- or bilateral cleft upper lip and jaw, cleft palate, partial aplasia of the nasal bone and upper jaw, bilateral dysplasia of the spine, and bent forefeet. All four fetuses had only partial development of the upper jaw and nasal bone. One fetus from this dam survived - all others died within two hours.

Under the conditions of this study in this strain of rabbit, HWA 486 was toxic to the dams at doses greater than 10 mg/kg. The drug was both lethal and embryotoxic at ≥ 15 mg/kg.

Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Himalayan Rabbits (Effect on Morphological Development) (ICH 4.1.3)

Document 13563

NDA Vol. 1.42, pp. 5-11366 through 5-11484

Study N^o: 94.0111

Report N^o: 95.0205

Compound: HWA 486 (A 77 1486 A), Batch N^o L 029-1

Formulation: Daily suspensions prepared in potato starch mucilage

Route: Oral, once/day from Day 6 - Day 18 of pregnancy at 5 mL/kg body weight.

Food/ Water Consumption: Both ad libitum

Strain: Himalayan (Chbb:HM(SPF), 6-10 months old, 2.704 ± 0.175 kg mean body weight.

Number of Animals: 20 mated ♀/group

Dosages: Group 1: 0 (5 mL vehicle) Group 2: 1 mg/kg Group 3: 10 mg/kg

Vehicle: Potato starch mucilage (20 g/L distilled water)

Study Site: Pharma Development, Corporate Toxicology, Hoechst AG, Frankfurt/Main

Date: June 6, 1994 - April 24, 1995

GLP/QAU Statements: Both present and signed.

The purpose of this study was to identify the maximum dose of the drug that was tolerated by dams and their conceptuses when administered orally to pregnant Himalayan rabbits. The study was conducted, as stated, in compliance with the ICH-Tripartite "Guideline on Detection of Toxicity to Reproduction for Medicinal Products" - June 23, 1993.

The low dose was expected to be nontoxic to the dams and conceptuses but to be in the therapeutic range for humans. Animal behavior and general physical condition were assessed daily. Food consumption was monitored once a week. Body weight development was determined twice a week. All females were killed Day 29 of pregnancy, cesarean sectioned, and

autopsied. Dams were dissected and macroscopic examination conducted on their organs.

RESULTS

- no drug related mortality to dams - 1 ♀ G3(10 mg/kg) killed D7 due to loss of wt 1st week-
- slight decrease in food consumption in 10 mg/kg dams-
- slight decrease (2%) in body weight D6 to D10 in 10 mg/kg dams-
- 1 ♀ G3 (10 mg/kg) not pregnant - 1 abortion in control group-
- no pathological changes in organs of dams of all groups-
- lower mean placental weights at 10 mg/kg - G1 (5.47 g), G2 (5.39 g), G3 (4.86 g)-
- normal development in live fetuses (body weight and crown/rump length) in both drug groups - all survived after 24 hr incubation-
- 1 fetus (10 mg/kg) with decreased body weight - sex ratio balanced in all groups-
- post-implantation loss % mean: G1 (4.28), G2 (9.33), G3 (11.75)-
 - early intrauterine deaths: DR - G1 (5), G2 (10), G3 (12)-
- 24 hour survival rate was 100% in all groups-
- epactal bone between both parts of nasal and frontal bone of 2 fetuses G1 and 1 fetus G3-
- diaphragmatic hernia in 1 fetus G2-
- blood in brain lateral ventricles (1 fetus) or in orbital region (2 fetuses of 2 litters) in G3-
- distended right ureter in 1 fetus and enlarged urinary bladder in 1 fetus G3-
- fused dysplastic sternbrae G3 (**17.2%), control (1.6%), low dose (3.3%) - indicated as "symptoms of embryotoxicity."-
- increase in number of fetuses/litter with blood in pericardium in G2 and G3

RESULTS DURING GESTATION AND AT CESARIAN SECTION

(From Vol. 1.42, pp. 5-11404 and 5-11405)

DOSE (mg/kg)	0	1	10
N° OF PREGNANCIES (total)	20	20	18
N° OF ABORTIONS (total)	1	0	0
N° OF FEMALES WITH PREMATURE DELIVERY (total)	0	0	0
N° FEMALES AT TERM WITH INTRAUTERINE DEATHS ONLY (total)	0	0	0
N° FEMALES AT TERM WITH LIVE FETUSES (total)	19	20	18
Corpora Lutea total (mean)	143 (7.5)	149 (7.5)	128 (7.1)
Implantations total (mean)	118 (6.2)	123 (6.2)	118 (6.6)
PRE-IMPLANTATION LOSS % (mean)	17.48	17.54	7.40
POST-IMPLANTATION LOSS % (mean)	4.28	9.33	11.75
Early intrauterine deaths total (mean)	5 (0.26)	10 (0.50)	12 (0.67)
% of Implantations (mean)	4.28	7.79	11.05
Late intrauterine deaths total (mean)	0 (0.00)	2 (0.10)	1 (0.06)
% of implantations (mean)	0.00	1.55	0.69
Total intrauterine deaths total (mean)	5 (0.26)	12 (0.60)	13 (0.72)
Live fetuses total (mean)	113 (5.9)	111 (5.6)	105 (5.8)

SUMMARY OF MAJOR FINDINGS IN LIVE FETUSES

(From Vol. 1.42, pp. 5-11408 to 5-11413)

DOSE (mg/kg)	0	1	10
EXTERNAL / VISCERAL DEFECTS OBTAINED AT AUTOPSY			
N° OF FETUSES EXAMINED / N° OF LITTERS EXAMINED	62*/19	60/20	58/18
SKULL			
Epactal bone between both parts of nasal/frontal bone	2/1	0/0	1/1
DIAPHRAGM			
Diaphragmatic hernia	0/0	1/1	0/0

Other defects obtained at autopsy that were considered variations or minimal follow:

- blood in pericardium: G1 (1/1), G2 (4/4), G3 (2/2)-
- lung- lobus inferior medialis - aplasia or lobus inferior medialis and lobus inferior sinister - completely fused or lobus superior sinister - bipartit: G1 (2/1), G2 (1/1), G3 (3/3)-
- blood in abdominal cavity: G1 (1/1), G2 (4/3), G3 (1/1)-
- pelvis distended - right or bilateral: G1 (1/1), G2 (0/0), G3 (3/2)-
- non-ossified or weakly ossified sternebra: G1 (17/9), G2 (17/9), G3 (22/12)-
- sternebra fused, dysplasia: G1 (1/1), G2 (2/2), G3 (10**/8**)-
- extra rib at 7th cervical vertebra - short and/or normally long - uni or bilateral: G2 (2/2), G3 (2/2)-
- enlarged stomach, taut with soft mass, displaced dextrad, transverse position: G1 (1/1), G2 (3/3)-

At 1 and 10 mg/kg, intrauterine development was not impaired. During the first week of treatment, a slight reduction in food consumption and body weight occurred in the dams dosed at 10 mg/kg. Fetuses from both drug groups showed normal development in terms of fetal weight and crown/rump length. Fused and dysplastic sternebrae in G3 were above the upper limit of historical controls. Other fetal defects reported in G2 and G3 that were slightly above study control include blood in pericardium, 2) aplasia or fusion of lung lobes, 3) distended right or bilateral pelvis, 4) non-ossified or weakly ossified sternebra, 5) fused, dysplasia of sternebra, 6) extra rib at 7th cervical vertebra or short or normally long rib, 7) blood in region of orbital cavity (G3 only), 8) blood in lateral ventricles (G3 only), 8) blood in pericardium, and 9) enlarged stomach increase in G3.

Under the conditions of this study, oral administration of HWA 486 at 1 mg/kg was not toxic to dams or to their fetuses. At 10 mg/kg the drug was slightly toxic to the dams. We would agree that 1 mg/kg was a tolerated dose in this strain of rabbit and their conceptuses.

- G4 food consumption ↓ significantly (49%) W2-
- G3 slight ↓ (4.4%) body weight gain D21 - G4 ↓ 8.9% D17 and * ↓ 24.3% D21-
- pregnant 19/20G1, 18/20G2, 19/20G3, 19/20G4-
- ♀ with dead conceptuses only G1(0), G2(0), G3 (1), G4 (5)-
- ♀ at term with live pups G1 (19), G2 (18), G3 (18), G4 (14)-
 - mean implantations G1 (13.8), G2 (13.9), G3 (12.8), G4 (13.3)-
 - mean live pups G1 (13.2), G2 (12.8), G3 (12.2), G4 (8.3, significant ↓)-
 - birth index % G1 (95.7), G2 (91.6), G3 (95.7), G4 (65.2, significant ↓)-
 - total dead pups (mean) 1G1 (0.05), 1G3 (0.06), 36G4 (2.57)-
 - % of implantations G1 (0.4), G3 (0.4), G4 (17.9, significant ↓)-
 - no effect on duration of pregnancy-
 - balanced sex ratios in all groups-
- no compound-related gross organ changes-
- enlarged spleens in dams 11 G4-

These results during pregnancy and at birth are indicated in the following table.

RESULTS DURING PREGNANCY AND AT BIRTH
(From Vol. 1.42, p. 5-11539)

DOSE (mg/kg)	0	0.40	1.25	4.00
NUMBER OF				
PREGNANCIES (total)	19	18	19	19
INTERCURRENT DEATHS (total)	0	0	0	0
FEMALES WITH DEAD CONCEPTUSES ONLY (total)	0	0	1	5
FEMALES AT TERM WITH LIVE PUPS (total)	19	18	18	14
Implantations total (mean)	262 (13.8)	251 (13.9)	230 (12.8)	186 (13.3)
Live pups total (mean)	251 (13.2)	230 (12.8)	220 (12.2)	116* (8.3)
Birth index % mean	95.7	91.6	95.7	65.2*
Dead pups total (mean)	1 (0.05)	0 (0.00)	1 (0.06)	36 (2.57)
% of implantations mean	0.4	0.0	0.4	17.9*
Sex of live pups ♂	51.8	48.7	43.2	53.4
Superumerary implantation sites total (mean)	10 (0.53)	21 (1.17)	9 (0.50)	34 (2.43)
% of implantations mean	3.9	8.4	3.9	16.9
GESTATION LENGTH mean	22.4	22.4	22.4	22.6

* significantly higher or lower than control

Abnormalities in pups at birth and to weaning

- number of litters G1 (19), G2 (18), G3 (18), G4 (14)-
- G4 extreme birth weight decreases - 39% ↓ at birth and 60% ↓ at D7 - G3 ↓ 40% D14-
- total live pups at birth (mean) G1 251(13.2), G2 230 (12.8), G3 220 (12.2), G4 * 116 (8.3)-
- total pups D14 (mean) G1 246 (12.9), G2 214 (11.9), G3 58 (3.2), G4 0 (0.0)-

Examination during lactation period - number of pups/litters affected (week)

- G1 1/2(1) poor suckling , 13/1(2-3) impaired coat growth + 1/1(1) hematoma on neck-
- G2 15/6(1-2) poor suckling, 32/4(2-3) yellow colored skin, 45/4(3) impaired coat growth, 10/1(2) abnormal gait, 1/1(3) corneal opacity/no pupillary reflex OD, 8/1(2) forepaws bent

- laterad, 1/1(3) extravasation of OD (no pupillary reflex)-
- G3 92/18(1-3) poor suckling, 80/14(1-3) pale forepaws, 22/2(1) pale skin, 11/12(2-3) yellow colored skin, 87/12(2-3) impaired coat growth, 150/15(1-3) abnormal gait and posture, 1/1(1) forepaws bent mediad-
 - G4 36/7(1) poor suckling, 8/2(1) pale forepaws, 1/1(1) pale skin, 18/2(1) abnormal gait, 29/5 malformations of paws/toes, 52/8 shortened/kinked tails, 3/2 tail tips missing-
 - external genital organ development not possible in G3 and G4 due to mortality - G2 development similar to control-
 - no data on function tests (hearing, vision, righting reflex) for G3 and G4 due to mortality - G2 function tests similar to control-
 - no data on behavior tests (trainability, memory, retrainability, motor activity, coordination, sense of balance) for G3 and G4 due to mortality - G2 appeared to be very similar to controls-

Fertility of the F1 generation (study continued with 19/sex G1 and 17/sex G2)

- unremarkable body weight of dams during pregnancy - G2 slightly higher than control-
- 1G2 not pregnant-
- duration of pregnancy not impaired in controls or G2-
- G1 and G2 littered normal developed live pups - 1 dead pup in G1, none in G2-

Autopsy results for pups of the F1 generation (killed after the function tests)

- corneal opacity 1G1 ♀ pup - extravasated eyes 1G1 ♀ pup-
- slight/moderate dilatation of renal pelvis 7G1 ♀ and 1G2 ♀-

Autopsy of parent animals of F1 generation

- no macroscopic changes in internal organs of ♂G1 and ♂G2-
- ♀ G2 which could not be mated more than once due to metestrus had a cyst with red fluid and an abscess in left uterus horn-
- 1 G1 female with dilated right renal pelvis-

The 4 mg/kg dose was toxic to the dams, producing diarrhea, weight reduction, reduced food consumption, early embryonic deaths, and mortality. The embryonic mortality seems to be due to the toxicity seen in the dams. Live pups from these dams had imperfect (bent) paws and abnormal gait. At autopsy, with the exception of enlarged spleens in the 4 mg/kg group, no compound-related changes developed in the internal organs of these dams. Birth weight and survival were significantly reduced in pups from the 1.25 and 4 mg/kg groups. Both groups had increasing numbers of pups and litters with bent paws. Yellow colored or pale skin occurred in pups of the treated dams during the lactation period. No adverse effects on vision, hearing, motor activity, trainability, or balance was reported for F1 pups in the 0.40 mg/kg group. The mean number of live pups in the 0.40 mg/kg lactation group began showing a downward trend as the days increased. These pups developed normally, but had poor suckling, yellow colored skin, impaired coat growth, abnormal gait/posture, and bent forepaws. This was marginal in terms of observed toxicity. No impairment was reported in the fertility or duration of the pregnancy for

the F1 generation. Pups of the F2 generation showed no outward abnormalities. The low dose of 0.4 mg/kg did not appear to be a NOEL dose.

Toxicokinetics of A77 1726 Following Administration of HWA 486 in an Additional Study Designed to Evaluate the Toxicokinetics in an Oral Rabbit Embryotoxicity Study.

Document 16702

NDA 20-905, Vol. 1.43, pp. 5-11697 to 5-11720

Study N^o: 97.0124

Compound: HWA 486

Formulation: Daily suspensions prepared in potato starch mucilage.

Route: Oral, once/day from Day 6 to Day 18 of pregnancy at 5 mL/kg body weight.

Food/Water: Both ad libitum.

Strain: Himalayan (Chbb:HM(SPF), 6-10 month old, 2.704±0.175 kg mean body weight

Number: 5/group

Dosages: 0, 1, 10 mg/kg

Vehicle: Potato starch mucilage (20 g/L in distilled water)

Study Site: Hoechst-Marion Roussel Drug Development, Walton, Milton Keynes, England

Date: October 14, 1997

GLP/QAU Statements: Both present and signed.

This study documents the toxicokinetics (C_{max} , t_{max} , AUC) of the major metabolite, A77 1726, in rabbit plasma. The data were obtained from a toxicokinetic addition to the oral rabbit embryotoxicity study (Document 13563, p. 14). Blood plasma concentrations of A77 1726 were determined by on D6 and D16 at 1, 2, 5, 8, and 24 hours post dose. The limit of quantification for the assay was 0.1 µg/mL.

RESULTS

Summary of the Toxicokinetics of A77 1726 in Rabbit Plasma Following Sampling in the Oral Rabbit Embryotoxicity Study

(Table 1, p. 5-11708)

Dose HWA 486 (mg/kg)	Day 6 ¹			Day 16 ²		
	C_{max} (µg/mL)	t_{max} (h)	AUC (µg·h/mL)	C_{max} (µg/mL)	t_{max} (h)	AUC (µg·h/mL)
Control	ND ³	ND	ND	ND	ND	ND
1	4.10	1.0	52.5	4.25	5.0	32.7
10	61.6	2.0	1052	101	1.0	1717

¹ Day 6 refers to day 6 of pregnancy, i.e., first day of dosing with HWA 486.

² Day 16 refers to the 16th day of pregnancy - the final day of dosing (i.e. the 11th dose)

³ ND = not detected

Values for t_{max} ranged over 1 to 5 hours and did not relate to dose levels or to the number of doses. Both C_{max} and AUC increased with increasing dose, but were not dose proportional. Following 11 doses (D16) of 10 mg/kg, C_{max} increased about 1.6 times and AUC values increased slightly over ± 6 times. Group 2 plasma concentrations ranged $\mu\text{g/mL}$ during the 24 hour Day 6 analysis and from 0.14 to 4.54 $\mu\text{g/mL}$ during the 24 hour Day 16 analysis. Respective values for Group 3 were 17.8 to 66.7 $\mu\text{g/mL}$ Day 6 and 14.3 to 108 $\mu\text{g/mL}$ Day 16.

Ames Test (Salmonella/Mammalian-Microsome Mutagenicity Test-Standard Plate Test) and Prival Modification (Salmonella/Mammalian-Microsome Mutagenicity Test-Preincubation Test.

Document 8407

NDA 20-905, Vol. 1.43, pp. 5-11721 to 5-11755

Study N^o: 88.0438

Compound: A77 1486, Batch 001-b

Formulation: Solution in DMSO

Route: Plate assay.

Strains: TA100, TA1535, TA1537, and TA98 of *Salmonella typhimurium*

Number: Three plates /dose level.

Dosage: 0, 0.8, 4, 20, 100, 500, 2500 $\mu\text{g/plate}$ \pm metabolic activation (Aroclor induced Sprague Dawley rat liver S9).

Controls:

(-) S9

(+) S9

Na azide: TA100 and TA1535

Benzo[a]pyrene: TA98, TA100, TA1535, TA1537

9-Aminoacridine with TA1537

2-Aminoanthracene: TA98, TA100, TA1535, TA 1537

2-Nitrofluorene with TA98

Benzidine and Congo Red: TA98 (Prival test)

Vehicle: DMSO

Study Site: Pharma Research Toxicology and Pathology, Hoechst AG, Frankfurt

Date: July 15, 1988

GLP/QAU Statements: Both present and signed.

A77 1485 was toxic to the strains at ≥ 500 $\mu\text{g/plate}$. No two fold increase in the number of revertants was seen in the presence or absence of S9 in any of the tester strains. All controls gave the expected changes in the number of revertants. In the Prival test [Mut. Res., 103-106 (1982)], in which a preincubation is done with hamster S9 supplemented with flavin mononucleotide, no significant increases occurred in the number of revertant colonies. HWA 486 was, therefore, not mutagenic in the standard Ames plate test.

A Mutagenicity Screening of HWA 486 in Bacteria (Ames Test).

Report 05/81

Document 8475

NDA Vol. 1.43, pp. 5-11756 to 5-11768

This appears to be an earlier Ames assay done in January 1981 in which HWA 486, Batch N^o op. IV at 4 µg - 2500 µg was evaluated with Salmonella typhimurium tester strains TA98 TA100, TA1535, TA1537, TA1538, and E. coli WP2 uvrA. S9 was prepared from Aroclor induced rat liver. Positive controls were 9-aminoacridine, 2-aminoanthracene, methylhydrazone derivative, streptocotocin (streptozotocin?), and ENNG. The negative control was DMSO. Each tester strain was evaluated with four plates in the absence and presence of S9. Under the limited conditions that were described, no mutagenic activity was seen.

Evaluation of A77 1486 in the Unscheduled DNA Synthesis Test in Mammalian Cells In Vitro.

Document 10326

NDA 20-905, Vol. 1.43, pp. 5-11769 to 5-11790

Report N^o: 90.1306Study N^o: 90.0514Compound: A77 1486, Batch N^o U 003, assays at 100.2%.Test Organism: A 549 N^o CCL 185 (permanent human cell line A 549, ATC N^o CCL 185)

Number: Six culture dishes were used for each dosage in two independent experiments.

Positive Controls: (- S9) 4-Nitroquinoline-N-oxide (1 µg/mL),
(+S9) Benzo(a)pyrene (10 µg/mL)

Dosages: 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 µg/mL

Cell Culture Medium: MEM with Hanks salts + 25 mM HEPES buffer

Vehicle: DMSO

Study Site: Pharma Research Toxicology and Pathology, Hoechst AG, Frankfurt.

Date: October 25, 1990 - November 6, 1990

GLP/QAU Statements: Both present and signed.

Culture dishes were seeded with 4×10^5 cells in Dulbecco's medium with 10% fetal calf serum and cultured at 37°C with 10% CO₂. A77 1486 was dissolved in DMSO, added to the plates, and the plates incubated for 3 hours in the absence or presence of rat liver homogenate (S9). The maximum concentration which did not precipitate in the medium was at 100 µg/plate. This was also the concentration producing cell toxicity. Tritiated thymidine was added to the cell culture after adding the test compound and S9. Following incubation, the medium was removed from cells and the cells rinsed twice, lysed, and the DNA isolated for counting. DNA concentrations were determined colorimetrically, and radioactive labeled thymidine incorporation into DNA was determined by liquid scintillation. Statistical evaluation was by the Student's t-test.

RESULTS

In both experiments the mean dpm/ μ g DNA did not significantly exceed the control means, either in the presence or absence of S9. At 10 μ g/mL, the counts began to fall off with or without activation. Both controls, benzo(a)pyrene and 4-nitroquinoline-N-oxide, were significant at $p < 0.0001$. It can be concluded that A77 1486 was inactive in the unscheduled DNA synthesis assay, either in the presence or absence of metabolic activation.

A77 1486 Detection of Gene Mutations in Somatic Cells in Culture HGPRT - Test with V79 Cells.

Document 8780

NDA 20-905, Vol. 1.43, pp. 5-11791 to 5-11814

Report N^o: 89.0099

Study N^o: 88.1076

Compound: A77 1486, Batch N^o U003

Formulation: Solution in DMSO

Cell Culture Medium: MEM with Hanks salts + 25 mM HEPES buffer

Test Organisms: V79 Chinese hamster lung fibroblasts

Number: 1×10^6 cells/flask

Dosages: 0, 50, 100, 150, 200, 220 μ g/mL

Negative Controls: a) untreated control, b) cultures treated with solvent (DMSO)

Positive Controls: (-S9) ethylmethansulfonate (EMS) and DMBA

Vehicle: DMSO

Study Site: Pharma Research Toxicology and Pathology, Hoechst AG, Frankfurt

Date: November 23, 1988 - January 25, 1989

GLP/QAU Statements: Both present and signed.

The S9 was prepared from Aroclor treated Sprague Dawley rats. Cultures were prepared and treated with A77 1486, incubated for 20 hours, then trypsinated, counted, diluted, and 400 cells per 25 cm² plated. The cells were then incubated 7 days with 6-thioguanine containing medium and the colonies stained with methylene blue and counted. Two independent experiments were carried with A77 1486, with and without metabolic activation. Dose levels were determined from two cytotoxicity studies that demonstrated significant cytotoxic effects at 250 μ g/mL to 1000 μ g/mL, the limit of solubility.

RESULTS

Under the conditions in which this study was carried out, A77 1486 did not significantly increase the number of mutant colonies or increase the frequency of mutation in the presence or absence of S9 metabolic activation. Positive controls EMS and DMBA produced mutant frequency increases of 12.2 times and 12.1 times above the solvent control, respectively.

A77 1486 (HWA 486) Micronucleus Test in Male and Female NMRI Mice after Oral Administration.**Document 10091****NDA 20-905, Vol. 1.43, pp. 5-11815 to 5-11840**Report N^o: 90.0781, Study N^o: 90.0491Compound: A77 1486 (HWA 486), Batch N^o U 003, purity stated as 100.2 %

Formulation: Suspension in starch mucilage.

Route: Oral, by gavage.

Food/Water: Both ad libitum

Strain: NMRI, Hoe:NMRKf (SPF71), 7 weeks old, ♂ 26-33 g, ♀ 22-28 g body weight.

Number: 5/sex/group

Dosages: 0, 150 (♂) mg/kg, 200 (♀) mg/kg, 50 mg/kg po cyclophosphamide positive control.

Vehicle: Starch mucilage

Study Site: Pharma Research Toxicology and Pathology, Hoechst AG, Frankfurt.

Date: June 25, 1990 - June 28, 1990

GLP/QAU Statement: Both present and signed.

Bone marrow was obtained from the proximal ends of the femora at 24, 48, or 72 hours following drug administration. Cells were smeared on slides and dried for 24 hours, then stained and 1000 polychromatic erythrocytes from each animal counted to determine the number of micronuclei. Statistical evaluation was by the method of Wilcoxon.

A preliminary study (150, 200, and 300 mg/kg) determined a dose of 150 mg/kg for males and 200 mg/kg for females was the maximum tolerated dose level. At 300 mg/kg (3/sex), one male died; the toxic signs were stilted gait, back-arched position, reduced spontaneous activity, piloerection, and trembling. At 200 mg/kg, (3/sex), one of the males died. Clinical signs noted were reduced spontaneous activity, red-brown lacrimation, forward movement in crawling posture, and narrowed palpebral fissures. The three males dosed at 150 mg/kg showed no clinical signs, and all survived.

RESULTS

- no signs of toxicity were reported-
- no animals died from treatment-
- no gross findings reported-
- no significant ↑ in micronucleated polychromatic erythrocytes in A77 1485 treated groups-
- positive control significant (*) ↑ in micronucleated polychromatic erythrocytes-

From the presented data, there was no significant increase in the number of micronucleated polychromatic erythrocytes. It can be concluded that A77 1486 was not mutagenic in the micronucleus test conducted under the OECD guideline N^o 474, 1983 and the conditions of this study.

Evaluation of A77 1486 (HWA 486) in the In Vivo Cytogenetic Test in Bone Marrow Cells of the Chinese Hamster - Chromosome Analysis.

Document 10470

NDA 20-905, Vol. 1.43, pp. 5-11841 to 5-11875

Report N^o: 91.0115, Study N^o: 90.0971

Compound: A77 1486 (HWA 486), Charge (lot) N^o U 003 of May 1988, purity 100.2%

Formulation: Suspension in potato starch mucilage.

Route: Oral, gavage

Food/Water: Both ad libitum

Strain: Chinese hamster, 10-14 weeks old, body weight ♂ 25-39 g, ♀ 22-34 g

Number of Animals: 5/sex/group for each killing time of 12, 24, and 48 hours

Dosage: 0, 60, 300, 600 mg/kg

Positive Control: Cyclophosphamide (Endoxan[®]) at 50 mg/kg - evaluated at 24 hr only.

Vehicle: Potato starch mucilage

Study Site: Pharma Research Toxicology and Pathology, Hoechst AG, Frankfurt

Date: December 17, 1990 - January 31, 1991

GLP/QAU Statements: Both present and signed.

This study was done to evaluate the mutagenic risk in Chinese hamsters from oral exposure to A77 1486. Dose selection for the high dose was based on 600 mg/kg as the maximum tolerated dose. The animals were killed at 12, 24, and 48 hours after dosing and the bone marrow removed from the femora. Following workup of the marrow, the cells were placed on slides, air-dried for 24 hours, stained (2% orcein solution), and 50 metaphases/animal were examined. Chromosomal aberrations were classified as gaps, iso-gaps, breaks, iso-breaks, fragments, iso-fragments, minutes, iso-minutes, deletions, iso-deletions, exchanges, dicentrics, chromosome disintegrations, ring formations, polyploidy, and multiple aberrations (metaphases with 5 and more aberrations). Only metaphases with 22 chromosomes were included in the analysis.

A preliminary study evaluated a single dose of 200, 500, 600, 800, 1000, and 1500 mg/kg. The 600 mg/kg of A77 1486 was shown to be the maximum tolerated dose. At 200 mg/kg, 3/sex survived without clinical signs of toxicity. At 500 mg/kg, 3/sex survived, but clinical signs of toxicity included narrowed and closed palpebral fissures and colorless lacrimation. At 600 mg/kg (3/sex), the highest dose evaluated in the preliminary study, all animals survived, but clinical signs noted were reduced spontaneous activity and colorless lacrimation. Higher doses resulted in increasing mortality.

The drug was considered mutagenic if 1) a significant increase is observed either in the enhancement of the rate clearly exceeding the control range or if the aberration rate is significant by the Fisher's exact test, and b) if there is a concentration related increase in the aberration rate. The drug is classified as non-mutagenic if no chromosomal aberrations were diagnosed.

RESULTS

PERCENTAGE OF METAPHASES WITH ABERRATIONS PER TRIAL GROUP

(From Vol. 1.43, p. 5-11860, Table 1)

Group	Dose mg/kg	Sacrifice Time (h)	Metaphases With Aberrations Including Gaps (%)		Metaphases With Aberrations Excluding Gaps (%)		Mitotic Index (X)		
			Males	Females	Males	Females	Male	Female	
(-) Control		0	12	0.8	1.2	0.0	0.0	7.56.6	
A77 1486	60	12	1.6	1.6	0.0	0.0	7.9	5.5	
A77 1486	300	12	1.2	2.4	0.0	0.4	6.2	5.7	
A77 1486	600	12	1.6	3.6	0.0	0.0	4.7	4.7	
(-) Control		0	24	2.4	1.6	0.4	0.0	9.6	8.7
A77 1486	60	24	2.4	0.8	0.0	0.4	7.5	9.0	
A77 1486	300	24	3.6	6.0	0.0	0.4	4.6	6.5	
A77 1486	600	24	3.2	8.4***	0.0	1.6	5.6	9.0	
Endoxan ^R	50	24	18.4#	16.8#	17.2	14.4	6.3	6.2	
(-) Control		0	48	0.4	1.6	0.0	0.0	3.0	2.8
A77 1486	60	48	5.6***	6.0	0.0	0.0	1.8	1.2	
A77 1486	300	48	3.2	4.8	0.0	0.0	1.8	1.8	
A77 1486	600	48	7.0***	6.8***	1.0	2.0	3.5	1.8	

*** p<0.001

p<0.0001

- one 600 mg/kg ♂ died prior to its scheduled 48 hour sacrifice-
- toxic signs observed were ↓ spontaneous activity, narrowed palpebral fissures, stilted gait, and tactile hyperesthesia-
- the mitotic index decreased slightly in the 12 hour female sacrificed group-
- significant ↓ (not DR) in the number of metaphases with aberrations including gaps occurred at 60 and 600 mg/kg in males at the 48-hour evaluation-
- significant ↓ (DR) in the 600 mg/kg treated females at the 24- and 48-hour evaluation-
- cyclophosphamide (Endoxan^R) ↓ aberrations at 24 hr, with (18.4%) and without (16.8%) gaps-

An increase in metaphases with aberrations in the 600 mg/kg female group at the 24- and 48-hour evaluation time. Significance was also observed at 48 hours in males dosed at 60 and 600 mg/kg, but these results were not DR over the treated group.

We note that the number metaphases per animal evaluated is usually done with more than 50.

4-Trifluoromethylaniline Study of the Mutagenic Potential in Strains of Salmonella Typhimurium (Ames Test) and E. Coli.

Document 12797

NDA 20-905, Vol. 1.43, pp. 5-11877 to 5-11904

Report N^o: 93.0546, Study N^o: 93.0390

Compound: 4-Trifluoromethylaniline, Batch N^o Wa 73, purity 99.7%

Route: Plate test

Positive Controls: (-) S9: Na azide, 9-aminoacridine, 2-nitrofluorene, MNNG
(+) S9: 2-aminoanthracene

Negative Controls: Solvent (0 µg/plate) and untreated control.

Strains: *S. Typhimurium* TA100, TA 1535, TA1537, and TA 98, and *E. Coli* WP2uvrA.

Number: Two independent experiments with three plates per experiment.

Dosages: 0, 4, 20, 100, 500, 2500, 5000 µg/plate

Vehicle: DMSO

Study Site: Pharma Development Central Toxicology, Hoechst AG, Frankfurt

Date: July 20, 1993 - September 15, 1993

GLP/QAU Statements: Both present and signed.

4-Trifluoromethylaniline, a metabolite of A77-1486 (HWA 486), was evaluated for mutagenicity in the Ames assay. The study was conducted in the presence and absence of rat liver homogenate S9. 4-Trifluoromethylaniline was toxic to the bacteria at ≥ 2500 µg/plate; it did not cause a precipitate at the high dose of 5000 µg/plate.

RESULTS

- (-) S9: 1 in revertant colonies with TA100 at 500 µg/plate in one experiment - 1 in revertant colonies with TA1535 at 100 and 500 µg/plate in both experiments - 1 in *E. coli* strain WP2uvrA at 500 and 2500 µg/plate in both experiments-
- (+) S9: 1 in revertant colonies with TA100 at 500 and 2500 µg/plate in one experiment and at 2500 µg/plate in the second experiment - 1 in revertant colonies with TA1535 at 100, 500, and 2500 µg/plate in one experiment and at 100, 500, 2500, and 5000 µg/plate in the second experiment - 1 in revertant colonies with *E. coli* strain WP2uvrA at 500, 2500, and at 5000 µg/plate-

From the results of this study it can be concluded that 4-trifluoromethylaniline was mutagenic to the above bacterial strains and to *E. coli* WP2uvrA in the presence and absence of S9. Increases were in excess of 2-fold in the mean number of revertants for (\pm S9) TA100, (\pm S9) TA1535, and (\pm S9) *E. coli* WP2uvrA.

4-Trifluoromethylaniline Ames Test (Salmonella/Mammalian-Microsome Mutagenicity Test-Standard Plate Test) and Prival Modification (Salmonella/Mammalian-Microsome Mutagenicity Test-Preincubation Test).

Document 8508

NDA 20-905, Vol. 1.45, pp. 5-11906 to 5-11940

This study was carried out according to the protocol (Document 8407, p. 26) described for the evaluation of A77 1486 (HWA 486). This study was conducted under GLP/QAU. S9 mixture was obtained from rat liver (Ames test) and from Syrian Golden hamster liver (Prival pre-incubation test supplemented with flavin mononucleotide). 4-Trifluoromethylaniline was

evaluated at 0, 4, 20, 100, 500, 2500, and 10000 $\mu\text{g}/\text{plate}$. A77 1486 was dissolved in 100 μL of DMSO. Because toxicity was observed at 500 and 2500 $\mu\text{g}/\text{plate}$ to most strains, and precipitation and/or incomplete or no bacterial lawns occurred at ≥ 500 $\mu\text{g}/\text{plate}$, the main study was evaluated at 20 to 500 $\mu\text{g}/\text{plate}$.

RESULTS

Dose dependent increases in the number of revertant colonies occurred at 300, 500, and 1000 $\mu\text{g}/\text{plate}$ with strains TA100 in the absence and presence of 30% rat liver S9 mix. Visible precipitation of the test compound on the plates was reported at ≥ 500 $\mu\text{g}/\text{plate}$. These increases were 2.1 to 5.2 times greater than the control values. Revertant colonies were also increased at 100 $\mu\text{g}/\text{plate}$ (2.2 times control value) and 300 $\mu\text{g}/\text{plate}$ (3.4 times control value) in the presence of Syrian golden hamster S9.

The number of revertant colonies per plate using TA1535 were increased from 2.5 to 5.2 times the control values at 100 $\mu\text{g}/\text{plate}$ and from 2.4 to 4.2 times the control values at 300 $\mu\text{g}/\text{plate}$ in the presence or absence of 30% rat liver S9 mix. Visible precipitation of the test compound on the plates was reported at 500 $\mu\text{g}/\text{plate}$. In the presence of 30% Syrian golden hamster liver S9, TA 1535 was increased 3.4 times above the control value at 100 $\mu\text{g}/\text{plate}$. Higher plate concentrations resulted in incomplete bacterial lawns.

No increases were reported for TA 1537 or TA 98, with or without 30% rat liver S9 mix or Syrian hamster S9 mix. The positive controls produced large expected increases in the number of revertant colonies. It can be concluded that 4-trifluoromethylaniline is mutagenic in the Ames assay in the presence and absence of metabolic activation.

4-Trifluoromethylaniline Detection of Gene Mutations in Somatic Cells in Culture HGPRT Test with V79 Cells.

Document 13344

NDA 20-905, Vol. 1.43, pp. 5-11941 to 5-11962

4-Trifluoromethylaniline, a major metabolite of A77 1486, was examined for mutagenic activity in V79 Chinese hamster lung fibroblasts. This study was conducted by Hoechst AG,

Frankfurt according a similar protocol (p. 21) described for the evaluation of the parent compound. GLP and QAU statements were provided and signed. The study was initiated on November 8, 1993 and terminated December 2, 1993. Dose levels of 10, 25, 50, 100, and 250 $\mu\text{g}/\text{mL}$ were tested without rat liver S9 and at 50, 100, 250, 500, and 750 $\mu\text{g}/\text{mL}$ with S9. Two independent assays were done with and without S9.

RESULTS

At 250 $\mu\text{g}/\text{mL}$ in the absence of S9, high levels of cytotoxicity were present, with survival at only 15.4% and decreasing further at the higher doses. In the presence of S9, almost

no cell survival occurred at ≥ 1000 $\mu\text{g/mL}$ - only 19.7% at 750 $\mu\text{g/mL}$. The results of the two independent assays, with and without S9, confirmed significant ($p \leq 0.05$) increases in the mutation rate. Mutation frequencies were increased ≥ 3 fold with and without S9.

These results show that 4-Trifluoromethylaniline is mutagenic in the HGPRT assay with V79 Chinese hamster cells when tested under the conditions used in this experiment.

4-Trifluoromethylaniline Micronucleus Test in Male and Female NMRI Mice after Intraperitoneal Administration.

Document 13086

NDA 20-905, Vol. 1.43, pp. 5-11964 to 5-11986

This study was similar to the micronucleus test carried out to evaluate the parent drug (Document 10091, p. 22) A77 1486. The present study was conducted by Hoechst AG, Frankfurt in August-October 1993. GLP and QAU statements were provided and signed. A suspension of 4-trifluoromethylaniline in potato starch mucilage was administered once to male and female mice at 80 mg/kg po. The animals (5/sex/kill) were killed at 12, 24, and 48 hours after metabolite administration and the number of polychromatic and normochromatic erythrocytes containing micronuclei counted. Endoxan^R (cyclophosphamide) was the positive control.

RESULTS

Cyclophosphamide produced significant ($p < 0.05$) increases in the number and percent of polychromatic erythrocytes with micronuclei in both sexes. The mutagenic index (erythrocytes with micronuclei in dose groups/erythrocytes with micronuclei in control) was 4.0 for cyclophosphamide at 24 hours, 1.0 for the control at 12, 24, and 48 hours, and 2.3 (12 hr), 0.2 (24 hr), and 1.3 (48 hr) for 4-trifluoromethylaniline. No significant increases in polychromatic erythrocytes with micronuclei were reported for 4-trifluoromethylaniline.

The results indicated that 4-trifluoromethylaniline was not mutagenic in the mouse micronucleus test.

4-Trifluoromethylaniline Chromosome Aberration In Vivo Cytogenic Test in Bone Marrow Cells of the Chinese Hamster.

Document 14161

NDA 20-905, Vol. 1.43, pp. 5-11987 to 11-12012

This study evaluated 4-trifluoromethylaniline in an in vivo chromosome aberration test. The assay was conducted according to the study on the parent compound (see Document 10470, p.). The study was conducted by Hoechst AG, Frankfurt during the period December 5, 1994 to February 7, 1995.

The assay contained 5 hamsters/sex/group. Bone marrow was isolated and worked up as

Test organism: Cells of Chinese hamster cell line V79.

Number: Two independent experiments using identical procedures.

Dosage: Without S9

With S9

At 18 hr: 0, 5, 10*, 50*, 100*, 250 µg/mL 0, 10*, 100, 250*, 500*, 650 µg/mL

At 28 hr: 0, 50, 100*, 250 µg/mL 0, 250, 500*, 650 µg/mL

* evaluated concentrations

Vehicle: DMSO

Controls: (-S9) EMS, (+S9) cyclophosphamide (Endoxan)

Study Site: Pharma Development Corporate Toxicology, Hoechst AG, Frankfurt

Date: January 24, 1994 - May 5, 1994

GLP/QAU Statements: Both present and signed.

This study was done to evaluate the potential of 4-trifluoromethylaniline to induce chromosome aberrations in V79 Chinese hamster cells in vitro. S9 was prepared from Aroclor induced rat liver. Concentrations were selected from the cytotoxicity assay. The mitotic index was determined in samples of 1000 cells. Cells were prepared at 18 and 28 hours. One hundred metaphases per experimental group and cell culture were examined. A one-sided Fisher Exact test was used to evaluate the results.

RESULTS

Summary results are in the following tables. Values in parentheses are from the repeat study.
(From Vol.1.43, Table 4, p. 5-12033)

TEST GROUP	DOSE µg/mL	S9	Fixation Interval (h)	Number of Cells Analyzed		Inc. Gaps Mean 1+2	% Aberrant Cells	
				1	2		Exc. Gaps Mean 1+2	Exchanges M 1+2
Solvent Control	0	-	18	100	100	2.5 (3.5)	2.0 (2.5)	0.0 (1.0)
4-TFMA	10	-	18	100	100	4.0 (5.0)	1.5 (1.0)	1.0 (0.0)
4-TFMA	50	-	18	100	100	6.5 (8.0)	3.0 (4.0)	0.5 (0.5)
4-TFMA	100	-	18	100	100	8.5 (9.0)	1.0 (3.5)	0.0 (0.0)
EMS	500	-	18	50	50	23.0 (26.0)	20.0 (23.3)	13.0 (14.7)
Solvent Control	0	+	18	100	100	1.5 (5.0)	0.0 (2.0)	0.0 (0.0)
4-TFMA	10	+	18	100	100	3.5 (2.5)	1.5 (0.5)	0.5 (0.0)
4-TFMA	250	+	18	100	100	9.0 (4.0)	5.5 (2.0)	2.0 (0.0)
4-TFMA	500	+	18	50	50	28.0 (33.0)	25.0 (27.0)	13.0 (10.0)
CPA	7.5	+	18	50	50	33.0 (56.0)	33.0 (56.0)	26.0 (51.0)
Solvent Control	0	-	28	100	100	4.5 (4.0)	2.5 (2.5)	0.0 (0.0)
4-TFMA	100	-	28	100	100	9.5 (11.5)	7.5 (9.5)	2.5 (3.0)
Solvent Control	0	+	28	100	100	2.0 (1.5)	1.0 (0.5)	0.0 (0.0)
4-TFMA	500	+	28	25	25 (50)	48.0 (36.0)	46.0 (33.3)	34.0 (24.0)

Without S9, high cytotoxicity was produced by 250 $\mu\text{g}/\text{mL}$; in the presence of S9, 650 $\mu\text{g}/\text{mL}$ was highly toxic. Cell toxicity was dose related. 4-Trifluoromethylaniline at 50 and 100 $\mu\text{g}/\text{mL}$ with the 18 hour preparation and in the absence of S9 produced significant increases in the number of aberrations, including gaps. At the 500 $\mu\text{g}/\text{mL}$ dose, aberrations were significant with and without gaps. These results were reproducible. At 18 hours with S9, significant increases occurred at 250 and 500 $\mu\text{g}/\text{mL}$ in the number of aberrations, with and without including gaps. In the second experiment, significance occurred only at 500 $\mu\text{g}/\text{mL}$. With the 28 hour preparation and 100 $\mu\text{g}/\text{mL}$ with or without S9, significant increases occurred in the number of aberrations including and excluding gaps. The reference mutagens showed positive increases in chromosome aberrations.

The results of this study confirm that 4-trifluoromethylaniline is mutagenic in the in vitro chromosome aberration assay with V79 Chinese hamster cells.

SUMMARY AND EVALUATION

The oral administration of HWA 486 (0, 0.4, 1.25, 4.0 mg/kg) in the Segment I study did not impair mating or pregnancy in rats, nor did it impair mortality, body weight or physical condition of the parent animals. Spermatogenesis, however, was not evaluated in this study. At 1.25 and 4.0 mg/kg, toxicity to the offspring occurred, causing weight loss, mortality, wavy/kinky tails, abnormal posture and limb movement, reduced suckling, and corneal opacity. A dose-related increase in intrauterine embryofetal mortality was also evident. Surviving F_1 offspring did not show impairment in fertility, pregnancy, parturition, or in postnatal development. At histopathology, no toxic effects were related to internal organs in any of the generations.

The Segment II dose-finding teratogenicity study was tolerated in Wistar rats (0, 5, 10, 15, 20, 30 mg HWA 486/kg) up to 15 mg/kg by the dams. Higher doses resulted in reduced food consumption and a lower body weight of the dams. At 5 mg/kg, no toxicity occurred to fetuses; however, higher doses produced significant malformations to the head, rump, vertebral column, and limbs of an increasing number of litters and fetuses.

The oral embryotoxicity study (Segment II) was repeated at 0, 1, and 15 mg/kg in Wistar rats. The 15 mg/kg dose was slightly toxic to the dams, producing a decrease in food consumption and a slight retardation in body weight. Fetuses from these dams had significantly lower fetal weight, crown/rump length, and the number of live fetuses were significantly reduced. Clear teratogenic results were observed at 15 mg/kg. Fifty-one percent of all fetuses in the nineteen high dose litters from this group developed malformations. The predominant malformations were concentrated in the brain (hydrocephalus), eyes (anophthalmic or microphthalmia), and cervical vertebral column. At the 1 mg/kg dose, no effects were seen on pregnancy or intrauterine development. No major findings in live fetuses were reported. The

minor irregularities in the skeleton and internal organs that occurred could be considered spontaneous.

A Segment II dose ranging study was carried out in Himalayan rabbits with 0, 5, 10, 15, 16, 20, 25, and 30 mg HWA 486/kg. Under the conditions of this study, ≥ 15 mg/kg were toxic to the dams, with higher doses becoming more lethal. Fetuses from dams dosed at 20 mg/kg showed no malformations, but fetuses from the 30 mg/kg dosed dam had malformations of the head. There were no fetuses to observe in dams dosed at 25 mg/kg, as none of the dams survived. Many of the findings seen in rats were also observed in these rabbits.

In the main rabbit oral embryotoxicity study (0, 1, 10 mg/kg), 1 mg/kg was not toxic to the dams or to their fetuses. The 10 mg/kg dose was slightly toxic to the dams, causing a decrease in food consumption and body weight. The mean total intrauterine deaths were increased at 10 mg/kg. Fetuses from this group also had a significant increase in fused dysplastic sternebrae.

Pre- and postnatal development, including maternal function in Wistar rats, was studied at 0, 0.4, 1.25, and 4.0 mg/kg. The high dose (4 mg/kg) was toxic to the dams, producing diarrhea and weight reduction. With the exception of enlarged spleens in the 4 mg/kg group, no compound-related changes developed in the internal organs of these dams. Birth weight and survival were significantly reduced in pups from the 1.25 and 4 mg/kg treated dams, and these doses were toxic to the intrauterine and postnatal development of the pups. Pups showed no adverse effects on vision, hearing, motor activity, trainability, or balance. Doses of 1.25 and 4 mg/kg were toxic to both dams and their offspring. Some pups from the 0.4 mg/kg group had poor suckling, yellow colored skin, impaired coat growth, abnormal gait, or bent forepaws. The fertility of the F1 generation was not impaired, and G1 and G2 had normal developed live pups. The 0.4 mg/kg dose did not appear to be at the NOEL. It appears the NOEL may be slightly less than 0.4 mg/kg.

Toxicokinetic data were obtained in Himalayan rabbits treated with HWA 486 from Day 6 to Day 18 of pregnancy. Values for t_{max} did not correspond to the number of doses administered or to the dose, and varied from 1 to 5 hours. Both C_{max} and AUC increased with doses of 1 and 10 mg/kg, but were not dose proportional. Following eleven doses, there was an increase of 1.6 fold in both C_{max} and AUC. Plasma concentrations for the 1 mg/kg dose varied from as high as 5.6 $\mu\text{g}/\text{kg}$ at hour 8 to 0.23 $\mu\text{g}/\text{kg}$ at hour 24 on Day 6. Day 16 values were 4.54 $\mu\text{g}/\text{kg}$ at hour 5 to 0.14 $\mu\text{g}/\text{kg}$ at hour 24. For the 10 mg/kg dose, plasma concentrations ranged from 17.8 $\mu\text{g}/\text{mL}$ to 66.7 $\mu\text{g}/\text{mL}$ on Day 6 of pregnancy and from 14.3 $\mu\text{g}/\text{mL}$ to 108 $\mu\text{g}/\text{mL}$ on Day 16 of pregnancy. A greater than proportional increase in the AUC and C_{max} was seen following repeat administration of 10 mg/kg.

Several studies were done to evaluate the mutagenic potential of HWA 486 and 4-trifluoro-methylaniline. HWA 486 was negative in the following studies: Ames assay, unscheduled DNA test, HGPRT test with Chinese hamster V79 cells, and the micronucleus test in mice. The in vivo chromosome aberration test using Chinese hamster bone marrow cells produced significant increases in aberrations in the 600 mg/kg treated female group at the 24- and

48-hour evaluation time and in the 48-hour male 60 and 600 mg/kg doses.

The metabolite, 4-trifluoromethylaniline, which has been detected at plasma levels of 8.1 ± 3.0 ng/mL in some patients receiving leflunomide, was evaluated and found positive in the Ames test, the HGPRT test with V79 Chinese hamster cells, and positive in the in vitro chromosome aberration tests with Chinese hamster V79 cells. It was negative in the mouse micronucleus test after intraperitoneal administration, and negative in the in vivo cytogenic test in bone marrow cells of Chinese hamsters. The labeling also indicated 4-trifluoromethylaniline was negative in the unscheduled DNA synthesis test in hepatocytes. This study was not included with the other mutagenicity studies, but was submitted as a reprint.

RECOMMENDATIONS:

Based on the reproductive and teratogenicity studies that have been reviewed in this report, there are no objections to the approval of this drug. It is recommended the pregnancy category not be designated as X, but rather as D or perhaps C. The sponsor should submit the GLP and QA statements for Document 10477 which were inadvertently deleted.

Almon W. Coulter, Ph.D.

Team Leader: _____
Andrea Weir, Ph.D., D.A.B.T.

cc:

NDA 20-905

HFD-550/Division File

/ACoulter

/AMukherjee

/KJohnson

/SCook

HFD-345

F/T by AWC: July 10, 1998

Reproductive Toxicity Assessment Committee (RTC) Report
Cover Sheet
Review of Reproductive Toxicity Studies

IND/NDA No.: 20-905

Drug Name: Leflunomide

CAS No.: 75706-12-6

Division Name/HFD No.: 550

Reviewer Name, Phone No.: Asoke Mukherjee, 301-827-2516

Sponsor: Hoechst Marion Roussel Inc.

Clinical Indications: Rheumatoid Arthritis

Drug Classification: Antimetabolite and Immunosuppressant

Date Submitted to RTC: July, 1998

Date of RTC Review:

Reviewing RTC Members:

Clearly state the basic question(s) you would like the RTC to answer.:

1. Leflunomide is an immunosuppressant and antimetabolite that inhibits pyrimidine synthesis. Reproductive safety data showed that the drug is teratogenic but did not impair fertility up to 4 mg/kg dose. Fertility at higher doses was not examined. The review of the reproductive toxicity studies are attached in Dr. Coulter's review dated July 14, 1998 and review dated March 1993.

Does the committee recommend pregnancy category X for Leflunomide?

Data clearly show that there is no effect on fertility at 4 mg/kg. Based on the segment I data, does the committee recommend that the label should indicate that the drug does not have any effect on fertility.

2. Long term safety studies in rats, mice and dogs showed oligospermia (pages 29, 44 pharm review NDA 20-905 dated July 1, 1998; page 19 review dated March 16, 1993), fibrosis of uterus (page 44 pharm review NDA 20-905 dated July 1, 1998), glandular polyps of uterus (page 28 pharm review NDA 20-905 dated July 1, 1998) and focal tubular atrophy in testes (page 16 pharm review NDA 20-905 dated July 1, 1998 and pages 17, 19 of pharm review dated March 16, 1993).

Considering these toxicities to the long term uses of the drug, does the committee recommend that the patients (males and females) who receive chronic treatment with Leflunomide should reduce the dose or washout the drug with charcoal or cholestyramine to the plasma level of 0.03 ug/ml before becoming pregnant. The proposal of the washout procedure has been indicated in the label and in volume 1.119 (two pages of the clinical report are attached). However, there are no preclinical data to support it.

3. Apart from pregnancy category whether there should be any other warning, box warning, precautions or advice for patients regarding reproduction?

The RTC's Responses to these questions are summarized below:

Question 1: The RTC discussed the pregnancy category issue for a considerable amount of time. The dose /exposure levels at which reproductive effects occurred in animal studies, the types of findings in the teratology studies, the intended patient population for leflunomide, risk versus benefit, and the comparison of leflunomide to other teratogenic drugs were discussed. At the end of the discussion, the Chair of the RTC, Mary Ellen McNerney, asked the members to state whether they felt the drug should be in category X or C. The vote was equally split among the 12 members of RTC. All members, including those who favored "C", felt that a box warning stating that the drug should not be used during pregnancy should be included in the label.

Regarding the fertility issue, the RTC felt that the available data support that leflunomide does not affect fertility in male or female rats. The RTC stated that the dose multiple (animal/human) for the fertility study should be included in the label.

Question 2: The RTC expressed concern about including the use of cholestyramine to hasten drug removal in the label without a specific human trial to demonstrate the efficacy of cholestyramine. The RTC also felt that a human mass balance study should be conducted, if one has not been conducted.

Question 3: The RTC recommended that the information relating to pregnancy be put in a box warning.

**APPEARS THIS WAY
ON ORIGINAL**

Memorandum for: Mary Ellen McNerney

From: Andrea Weir

Subject: Request for Evaluation of Segment 2 Reproductive Toxicity Data

SUMMARY: In March 1998, Hoescht Marion Roussel submitted NDA 20-905 for leflunomide to the Division of Anti-inflammatory, Analgesic, and Ophthalmic Drug Products. Leflunomide, an isoxazole immunomodulatory agent, is intended for use in the treatment of active rheumatoid arthritis. It inhibits de novo pyrimidine synthesis and has antiproliferative activity. The mode of action of leflunomide may be inhibition of dihydroorotate dehydrogenase. The plasma half-life of leflunomide in humans is 189 hours.

In accordance with regulatory guidelines, the sponsor submitted a Segment 2 reproductive toxicity study for rats and rabbits. A review of the rat study is attached. The sponsor used only three groups in this study, a control and two treatment groups (1 mg/kg and 15 mg/kg). Dams in the 15 mg/kg exhibited maternal toxicity (up to 11% decrease in body weight compared to the control group). As the data provided in the attached review demonstrate, fetal abnormalities (anophthalmia/microphthalmia in 29 fetuses/13 litters and internal hydrocephalus in 28 fetuses/15 litters) were observed in the 15 mg/kg group. The sponsor concluded that the 15 mg/kg dose is teratogenic. The 1 mg/kg dose had no effects on dams or fetuses. The systemic exposure (ug.hr/mL) in the 15 mg/kg group was one-tenth the maximum clinical exposure.

QUESTIONS:

1. Does the Committee agree with the sponsors assessment?
2. Does the Committee feel that maternal toxicity was in any way involved in the fetal effects?

Responses attached

Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Wistar Rats (Effect on Morphological Development) ICH 4.1.3

Document 13220.

NDA 20-905, Vol. 1.41, pp. 5-10874 to 5-11056.

Study N^o: 94.0110, Report N^o: 95.0246,

Compound: HWA 486 (A 77 1486 A), Batch N^o L 029-1

Formulation: Suspension prepared each day in starch mucilage.

Route: Oral, by stomach tube once/day at 5 mL/kg from 7th - 19th day of pregnancy.

Food/Water: Ad libitum.

Strain: Wistar, approximately 65-75 day old, 189.3 ± 11.3 g mean body weight

Number: 22 mated ♀/group

Dosages: Group 1 0 (5 mL starch mucilage/kg)

Group 2 1 mg/kg

Group 3 15 mg/kg

Vehicle: Starch mucilage (20 g potato starch/L distilled water)

Study Site: Pharma Development, Corporate Toxicology, Hoechst AG, Frankfurt/Main

Date: February 25, 1994 - July 6, 1995

GLP/QAU Statements: Both present and signed.

This study was conducted to identify the maximum dose of HWA 486 (A 77 1486 A) that can be tolerated by dams and their conceptuses when given orally to pregnant rats from pregnancy Day 7 to pregnancy Day 19, the sensitive period of organogenesis. On the 21st day after mating the rats were killed and the fetuses delivered by caesarean section. The examinations included autopsy and skeleton analysis in about 50% and body-cross sections after Wilson in about 50%.

RESULTS

- no impairment of dam behavior or general physical condition-
- food consumption significantly ↓ from control in 15 mg/kg dams D7-14 (10.8%) and D14-D21 (6.3%)-
- body weight of dams ↓ 7.1%* D14, 10.3% D17, 11.7% D21 at 15 mg/kg-
- at autopsy of dams:
 - 1G2 with light-brown cortex at cranial and caudal pole of kidney-
 - 1G1 and 3G2 with dilated renal pelvis (one or both)-

Gestational and Caesarean Section Results
(From Vol. 1.41, p. 5-10912 and p. 5-10913)

	GROUP 1 0 mg/kg	GROUP 2 1 mg/kg	GROUP 3 15 mg/kg
PREGNANCIES TOTAL	20	20	20
FEMALES AT TERM WITH LIVE FETUSES TOTAL	20	20	20
CORPORA LUTEA TOTAL MEAN (S.D.)	272 13.6 (1.7)	283 14.2 (1.7)	273 13.7 (4.0)
IMPLANTATIONS TOTAL MEAN (S.D.)	251 12.6 (2.7)	267 13.4 (1.5)	262 13.1 (3.4)
PRE-IMPLANTATION LOSS % MEAN	7.7	5.25	5.87
POST-IMPLANTATION LOSS % MEAN	8.68	5.23	29.20
% OF IMPLANTATIONS	8.68	4.89	25.90*
LIVE FETUSES TOTAL MEAN (S.D.)	231 11.6 (3.1)	253 12.7 (1.6)	182 ^b 9.1 (3.4)

*Significantly higher than control

^bSignificantly less than control

- corpora lutea and implants similar to control-
- mean loss of early implantations- G1 (8.68%), G2 (4.89%), G3 (25.9%)*
- % of implantations- (mean post-implantation loss % minus % mean of implantations) G1 (0.00%), G2 (0.33%), G3 (3.30%)-
- live fetuses reduced in 15 mg/kg due to embryofetal deaths-
- conceptuses undergoing resorption and dead fetuses ↑ at 15 mg/kg (*)

RESULTS IN LIVE FETUSES AT CAESAREAN SECTION
(From Vol. 1.41, p. 5-10914)

	GROUP 1 0 mg/kg	GROUP 2 1 mg/kg	GROUP 3 15 mg/kg
NUMBER OF FETUSES TOTAL MEAN (S.D.)	231 11.6 (3.1)	253 12.7 (1.6)	182* 9.1 (3.4)
% OF IMPLANTATIONS MEAN	91.32	94.77	70.80*
CROWN/RUMP LENGTH MM MEAN (S.D.)	35.2 (1.3)	34.9 (1.3)	32.3 (1.4)*
PLACENTAL WEIGHT, GRAMS MEAN (S.D.)	0.47 (0.05)	0.44 (0.04)	0.36 (0.04)*
FETAL WEIGHT, GRAMS MEAN (S.D.)	3.4 (0.3)	3.4 (0.2)	2.8* (0.2)
N° OF LITTERS	20	20	19

*Significantly less than control

- sexes were evenly balanced-

- 92 fetuses from the 19 litters in G3 exhibited the following malformations-
 - brain (internal hydrocephalus) - eyes (anophthalmia/microphthalmia, oval-shaped lens), cervical vertebral column (aplasia, partial aplasia, dysplasia, fusions)-
 - many combined malformations-
- other G3 malformations - edema in subcutis, encephalocele near frontal/parietal bone, asymmetry of viscerocranium, brachygnathia inferior, partial fusion of both rami of mandible, diaphragmatic hernia and scoliosis of thoracic vertebral column-
- delayed skeletal ossification at 15 mg/kg - other findings are indicated in the following summary table-

SUMMARY OF MAJOR FINDINGS IN LIVE FETUSES
(From Vol. 1.41, pp. 5-10916 to 5-10925)

DOSE (mg/kg)	0	1	15
EXTERNAL/VISCERAL DEFECTS OBTAINED AT AUTOPSY			
Nº OF FETUSES EXAMINED / Nº OF LITTERS EXAMINED	120/20	131/20	96/20
EXTERNAL			
Retarded fetuses	1/1	0/0	12/10
BODY			
Edematous fetus	0/0	0/0	1/1
HEAD			
Cranium protruding in region of frontal or parietal bone	0/0	0/0	3/2
Facial part of the skull - asymmetric	0/0	0/0	1/1
JAW			
Brachygnathia inferior	0/0	0/0	1/1
EYE			
Anophthalmia or microphthalmia - uni- or bilateral	0/0	0/0	29/13
BRAIN			
Hydrocephalus internus	0/0	0/0	28/15
DIAPHRAGM / LUNG / LIVER			
Diaphragmatic hernia - left lung - left half of lungs displaced - cranial lung - lobus sinister - reduced in size, liver - lobus sinister and lobus accessorius displaced to thoracic cavity, liver - lobus dexter, lobus dexter accessorius and lobus caudatus - fused	0/0	0/0	1/1
EXTERNAL / VISCERAL AND SKELETAL DEFECTS			
Retarded fetus; brain - skull, forelimb, forepaw multiple defects	0/0	0/0	1/1
EYE			

DOSE (mg/kg)	0	1	15
Anophthalmia - right, microphthalmia - left, orbit reduced in size - bilateral; skull/cervical vert. arch - dysplasia 2nd - right, fused 3rd and 4th - ventrad - left; brachygnathia superior; jaw - mandible fuses - partly; cheilognathopalatoschisis - left; facial part of the skull - asymmetrical; encephalocele in region of parietal bone, nasal bone - dysplasia, frontal, parietal and interparietal bone - only marginally preformed	0/0	0/0	1/1
SKELETAL DEFECTS			
N° OF FETUSES EXAMINED / N° OF LITTERS EXAMINED			
SKULL			
Orbit - reduced in size - uni- or bilateral	0/0	0/0	25/13
Exoccipital bone - dysplasia - uni- or bilateral	0/0	0/0	9/4
INCISOR / JAW			
Mandible fuses - partly	0/0	0/0	2/2
SKULL / CERVICAL VERT. ARCH			
Os exoccipita bone fuses with 1st cervical vertebra; anlage of only 4, 5, or 6 cervical vertebral arches only dorsal part developed, aplasia, partial aplasia, dysplasia, fused	0/0	0/0	39/18
VERTEBRAL COLUMN / RIB			
Scoliosis in region of thoracic vertebrae - sinistrad thoracic vert. Centra - dislocated, fragmented longitudinally displaced 4th, 7th, 11th, 12th fused vert. Arches - 3rd, 4th cervical arch - right fused vert. Arches - 5th, 6th thoracic arch - right rib - vestigial 7th - right fused ribs - 10th, 11th proximal part - right	0/0	0/0	4/4
EXTERNAL / VISCERA DEFECTS OBTAINED AT BODY CROSS-SECTION EXAMINATION			
N° OF FETUSES EXAMINED / N° OF LITTERS EXAMINED			
	111/20	122/20	86/20
EXTERNAL			
Retarded fetus	1/1	0/0	11/9
HEAD			
Encephalocele in region of frontal and parietal bone	0/0	0/0	1/1
JAW			
Brachygnathia inferior	0/0	0/0	1/1
EYE			
Anophthalmia or microphthalmia - uni- or bilateral	0/0	0/0	23/10
Lens dysplastic - oval shaped - left	0/0	0/0	1/1
BRAIN			
Hydrocephalus internus	0/0	0/0	31/14

At 1 mg/kg, no effects were seen on pregnancy or intrauterine development. Only minor irregularities in the skeleton or internal organs occurred at this dose and can be considered to be spontaneous. Results at 15 mg/kg, however, clearly revealed the teratogenic potential of this

drug, with 51% of all fetuses in the 19 litters developing malformations. The predominant malformations were concentrated in the brain (33% internal hydrocephalus), eyes (23% anophthalmia or microphthalmia), and 23% malformations in the cervical vertebral column. This dose was also slightly toxic to the dams.

**APPEARS THIS WAY
ON ORIGINAL**