

Vehicle (PBS) control, DX-88 23 mg/kg Lot No. 493034, 6.8 mg/kg Lot No. 110184, and 23 mg/kg Lot No. 110184 were administered intravenously once daily for 14 days.

Two females in the 23 mg/kg Lot No. 493034 group and two females in the 23 mg/kg Lot No. 110184 group were found dead during the study. Two TK animals, one with each lot, were not necropsied or microscopically evaluated. For the toxicology female that received Lot No. 493034, prostration and gasping were noted prior to death. For the two toxicology females found dead, microscopic findings included moderate congestion of the kidneys, liver, lungs and ovaries, mild congestion of the adrenal and pituitary, and vacuolation of cortical renal tubular epithelium. There were additional findings of minimal subacute inflammation and hemorrhage of the lungs and hemorrhage and minimal epidermal exudate at the injection site in one female of the 23 mg/kg Lot No. 110184. The cause of death was undetermined.

For surviving animals, transient hypoactivity was observed in high dose females of both Lots. Injection site findings observed for two 6.8 mg/kg Lot No. 110184 females and/or one 23 mg/kg Lot No. 110184 female included severe acute inflammation, surface exudate, necrosis of the tail, and/or severe epithelial hyperplasia.

Only the selected tissue/organs were microscopically evaluated in all control and high dose animals. All collected tissues/organs were microscopically evaluated only in n=1/sex/control or 6.8 mg/kg group in addition to the two found dead females. Therefore, the NOAEL could not be determined.

For the evaluated parameters, there appeared to be no difference in toxicity between the drugs from Lot No. 493034 and Lot No. 110184.

Study no.: (b) .446004

Volume #, and page #: Electronic submission

Conducting laboratory and location:

(b) (4)

Date of study initiation: November 26, 2005

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: DX-88,

DX-88 from original manufacturing process, in phosphate buffered saline (PBS), pH 7.0, Lot/batch No. 493034; purity, 95.2%, concentration=10.3 mg/mL,

DX-88 from current manufacturing process, in phosphate buffered saline (PBS), pH 7.0, Lot/batch No. 1100184, purity, 93.7%, concentration=10.8 mg/mL.

Methods

Doses: 0, 6.8 and 23 mg/kg, administered once daily for 14 days.

Species/strain: Rats / CrI:CD®(SD), from (b) (4)

Number/sex/group or time point (main study): See the table for Toxicology Groups below (inserted from page 27 of the report)

Route, formulation, volume, and infusion rate: The route of administration is subcutaneous. The stock test article was diluted in vehicle to achieve the desired concentrations. Dose volume is listed in the tables below.

Toxicology Groups (b) (4) -446004)

Group Number	Treatment	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Dose Level (mg/kg)	Number of Animals	
					Males	Females
1	Vehicle ^a	4.6	0	0	8	8
2	DX-88 Lot 493034	4.6	5.0	23.0	8	8
3	DX-88 Lot 1100184	4.6	1.48	6.8	8	8
4	DX-88 Lot 1100184	4.6	5.0	23.0	8	8

^a - Vehicle was phosphate buffered saline (PBS) for injection.

Satellite groups used for toxicokinetics: As shown in the tables below, n=8/sex/group for DX-88 treated groups were used for toxicokinetics evaluation.

Toxicokinetic Group: (b) -446004A)

Group Number	Treatment	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Dose Level (mg/kg)	Number of Animals	
					Males	Females
2A	DX-88 Lot 493034	4.6	5.0	23.0	6	6
3A	DX-88 Lot 1100184	4.6	1.48	6.8	6	6
4A	DX-88 Lot 1100184	4.6	5.0	23.0	6	6

^b - Groups 2A, 3A and 4A were dosed with the test article formulations in the same manner as Groups 2, 3 and 4, respectively. Data for toxicokinetic animals were collected in a separate computer protocol.

Age: Approximately 7 weeks at initiation of dose administration.

Weight: toxicology groups: 224-257 g for males and 159-183 g for females;

toxicokinetic groups: 223-269 g for males and 151-192 g for females;

Sampling times:

Observations and times:

Mortality: All animals were observed twice daily.

Clinical signs: For toxicology groups, animals were observed twice daily, at the time of dosing and approximately 1-2 hours post dosing. Detailed physical examinations were conducted on all animals weekly beginning 1 week prior to initial dosing.

Body weights: Individual body weights of all animals were recorded at least weekly beginning approximately 2 weeks prior to initial dosing.

Food consumption: Individual food consumptions were recorded weekly beginning approximately 2 weeks prior to initial dosing.

Ophthalmoscopy (toxicology groups): Ocular examinations were conducted prior to initial dosing, and at the end of treatment.

EKG: Not measured

Hematology and Clinical chemistry: Blood samples were collected at the scheduled necropsy.

Urinalysis: not conducted

Gross pathology (toxicology groups): A complete necropsy was performed on all surviving animals at the scheduled necropsy. Necropsy was also performed on the animals died early in the study.

Organ weights (toxicology groups): See "Histopathology Inventory" table.

Histopathology (toxicology groups): The tissues and organs listed in "Histopathology Inventory" table below were collected from all necropsied animals and placed in 10% neutral-buffered formalin except noted otherwise. Epididymides and testes were fixed in Bouin's solution, and the eyes with optic nerves were fixed in Davidson's solution. Gross lesions were examined for all necropsied animals. Injection sites were examined for the females from control, 23 mg/kg Lot No. 493034 and 23 mg/kg Lot No. 1100184 groups. Bone marrow smears were not placed in formalin and the slides were examined only if scientifically warranted. Thyroid with parathyroids were examined if in the plane of section and in all cases where a gross lesion was present.

Adequate Battery: Yes

Peer review: Yes (for two found dead animals and n=1/sex from control and 6.8 mg/kg dose groups.)

Toxicokinetics: Blood samples (~0.5 mL) were collected via retro-orbital sinus under anesthesia at 5, 15, 45, 180 and 360 minutes post dosing on Dose 1 (Day 0) and on Dose 14 (Day 13). The samples were analyzed for the concentration of DX-88 using a validated HPLC/MS method (Study No. AA16809-UXZ) by (b) (4)

Results

Mortality: Two females (No. 40546 from toxicology group, No. 40565 from TK group) in the 23 mg/kg Lot No. 493034 group were found dead on SD 13. Two females that received 23 mg/kg Lot No. 1100184 group were found dead as follows: on SD 9 (female No. 40585 from toxicology group) and on SD 0 (female No. 40577 from TK group). No observations for the found dead females in TK groups were reported. Prostration and gasping were noted in female No. 40546 while no significant observations were noted in another toxicology group female No. 40585. The congestion of the kidneys, liver, lungs and ovaries, and vacuolation of cortical renal tubular epithelium was noted in these two found dead females of toxicology group. Subacute inflammation and hemorrhage of the lungs and hemorrhage and epidermal exudate at the injection site was observed in the female No. 40585 of the 23 mg/kg Lot No. 110184. The microscopic evaluation of all collected tissues was performed on both found dead toxicology females and one each for the control and 6.8 mg/kg group males and females. However, for animals found dead, the causes of death were not evident based on the microscopic findings as well as a peer review of the microscopic data. All other animals survived to the scheduled necropsy.

Clinical signs: For the surviving animals, hypoactivity was observed within 30 min post dose during SDs 6-11 in 5 females/1 male of the 23 mg/kg Lot No. 493034 group and in 3 females of the 23 mg/kg Lot No 110184. There were no other test article-related clinical observations.

Body weights: There were no test article-related effects on body weights.

The mean body weights among control and treated groups were comparable. **Food consumption:** There were no test article-related effects on food consumption.

Ophthalmoscopy: There were no test article-related ophthalmic lesions observed.

EKG: not conducted.

Hematology: There were no test article-related alterations of measured parameters. There were no toxicologically significant differences in the measured parameters between the dose groups of 23 mg/kg Lot No. 110184 and 23 mg/kg Lot No. 493034. There were no significant test article-related effects on the measured APTT and PT values.

Clinical Chemistry: There were no significant test article-related adverse effects on the measured parameters. There were no toxicologically significant differences in the measured parameters between the dose groups of 23 mg/kg Lot No. 110184 and 23 mg/kg Lot No. 493034.

Urinalysis: not measured.

Gross pathology: There were no test article-related gross findings in the two found dead females of the toxicology groups and in the scheduled necropsied animals.

Organ weights: There were no test article-related effects on organ weights. The organ weights in dose groups of 23 mg/kg Lot No. 110184 and 23 mg/kg Lot No. 493034 were comparable. As shown in the table below, there were slight decrease in absolute and relative thymus weight (up to -31%) and slight increase in thyroid/parathyroid weights (up to +43%) in both female 23 mg/kg dose groups. No microscopic findings were associated with these slight organ weights changes in females and the similar trend was not observed in the males. Hence, these changes in females were not considered toxicologically significant.

Organ	Weight	Female groups			
		control	23 mg/kg Lot 493034	6.8 mg/kg Lot 110184	23 mg/kg Lot 110184
Thymus	Absolute (g)	0.65	0.49 (-25%)	0.52	0.45 (-31%)
	Ab/Body	0.35	0.28 (-20%)	0.29	0.25 (-29%)
	Ab/Brain	35.7	26.8 (-25%)	28.5	24.8(-31%)
Thyroid/Parathyroid	Absolute	0.13	0.15 (+15%)	0.14	0.17 (+30%)
	Ab/Body	0.007	0.009 (+29%)	0.008	0.10 (+43%)
	Ab/Brain	0.73	0.85 (+16%)	0.77	0.95 (+30%)

Histopathology: All the collected tissues/organs of the found dead females, and of one male (No. 40480) and female from control group, one male (No.40498) and one female (No. 40549) from 6.8 mg/kg (lot No. 1100184) dose group were processed and stained with hematoxylin and eosin for microscopic examination. In addition, the selected tissues including the heart, lung, brain, liver, and kidneys from all animals in the control, 23 mg/kg Lot No. 493034 and 23 mg/kg Lot No. 1100184 groups were process for microscopic examination.

For both of the two found dead females, as described in the section of "Mortality" above, histopathology findings included moderate congestion of the kidneys, liver, lungs and ovaries, mild congestion of the adrenal and pituitary, and vacuolation of cortical renal tubular epithelium. There were additional findings of minimal subacute inflammation and hemorrhage of the lungs and hemorrhage and minimal epidermal exudate at the injection site in one female of the 23 mg/kg Lot No. 110184. The causes of death could not be determined.

For the animals that survived to the scheduled necropsy, there were no significant test article-related microscopic findings of the selected tissues. Thus, no systemic toxicity of DX-88 was evident in these animals.

Noteworthy observations for the surviving animals were listed below. However, these findings were either in low incidence/severity, not observed in the opposite gender, or consistent with background lesions in rats, hence, were unlikely to be test article-related adverse effects.

Findings	Males				Females			
	Dose (mg/kg)	Ctl.	23 ^a	6.8 ^b	23 ^b	Ctl.	23 ^a	6.8 ^b
Number of animals	8	8	1	8	8	7	1	7
Kidney, tubular necrosis	0	0	0	0	0	0	0	1(3)
Kidney, hyperplasia transitional cells	0	0	0	0	0	1(3)	0	0
Kidney, acute inflammation	0	0	0	0	0	1(3)	0	0
Kidney, inflammation, chronic	0	0	1(4)	0	0	0	0	0
Liver, necrosis	0	0	0	0	0	0	0	1(1)
Lung, hyperplasia, bronchial-alveolar	0	0	0	1(1)	0	0	0	0
Lung, macrophages, alveolar	2(1)	1(1)	1(1)	1(1) 2(2)	1(1)	1(1)	1(1)	0
Heart, subacute inflammation	1(1)	2(1)	0	3(1)	0	0	0	3(1)

The number in the parentheses indicates the level of severity: 1=minimal, 2= mild/slight, 3= moderate. 4=severe; Ctl.= control,

a= Lot No. 439034, b= Lot No. 110184

Common injections site findings including inflammation and hemorrhage were observed, regardless of the treatment group while more severe injection site lesions including severe acute inflammation, surface exudate and necrosis of the tail in some sections were observed in 2 females in the 6.8 mg/kg Lot No. 110184 and 1 female in the 23 mg/kg Lot No. 110184 had, and severe epithelial hyperplasia was observed in 1 female in the 23 mg/kg Lot No. 110184.

Toxicokinetics: The summary of data was listed below (excerpted from page 34 of the report).

TOXICOKINETIC RESULTS						
Gender/ DX-88 (mg/kg/day)	DX-88 Results					
	AUC ₀₋₆ (µg·h/mL)		C _{max} (µg/mL)		t _{max} (min)	
	Day 0	Day 13	Day 0	Day 13	Day 0	Day 13
Males						
23.0 (Lot No. 493034)	20.9	27.4	25.5	52.9	15	5
6.8 (Lot No. 1100184)	8.90	11.3	17.1	25.7	5	5
23.0 (Lot No. 1100184)	33.0	33.5	65.1	52.6	5	5
Females						
23.0 (Lot No. 493034)	27.6	22.2	58.4	32.3	5	5
6.8 (Lot No. 1100184)	6.49	7.20	13.0	9.47	5	5
23.0 (Lot No. 1100184)	30.7	23.5	63.6	31.6	5	5

Study title: DX-88: 14-day Intravenous Toxicity Study in Cynomolgus Monkeys**Key study findings:**

Monkeys were administered IV doses of 0, 2.3, 6.8 and 23 mg/kg/day.

No significant test article-related toxicity was observed.

The NOAEL was identified at 23 mg/kg (AUC_{0-360 min} = 6361 µg.min/mL for males or 9212 µg.min/mL for females on Day 14).

Study no.: (b) (4) 3130

Volume #, and page #: Electronic submission

Conducting laboratory and location:

(b) (4)

Date of study initiation: December 15, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: DX-88 in PBS, pH 7.0, Lot # 00799DXP, purity, ~97.7%, concentration 10.1 mg/mL

Methods

Doses: 0, 2.3, 6.8 and 23 mg/kg, administered intravenously once daily for 14 consecutive days.

Species/strain: Cynomolgus monkeys from

(b) (4)

Number/sex/group or time point (main study): See the table below (inserted from page 2 of the report).

Treatment Groups	Dose Level (mg/kg)	Dose Conc. (mg/mL)	No. of Animals	
			Males	Females
1. Control *	0	0	3	3
2. Low Dose	2.3	1.0	3	3
3. Mid Dose	6.8	2.96	3	3
4. High Dose	23.0	10.0	3	3

Route, formulation, volume, and infusion rate: The route of administration is subcutaneous. The control/vehicle was 0.9% NaCl for Injection, USP. The stock test article was diluted in vehicle to achieve the desired concentration. Dose volume was 2.3 mL/kg for all animals.

Satellite groups used for toxicokinetics: N/A. TK samples were collected from the main study animals.

Age: 2.5-3 years old for males and 3-4 years old for females at the initiation of dosing.

Weight: 2.6 -3.1 kg for males and 2.6 - 3.1 kg for females.

Unique study design or methodology (if any): N/A

Observations and times:

Mortality: Mortality was checked once daily.

Clinical signs: Cage-side clinical observations were conducted twice daily in treatment and recovery phases. Detailed physical examinations were conducted on all animals once weekly.

Body weights: Individual body weights of all animals were recorded weekly beginning approximately 1 week prior to initial dosing. Terminal body weights (fasted) were recorded prior to the necropsy

Food consumption/appetite: Food consumptions were assessed qualitatively daily and recorded as part of the daily observations. Appetite was evaluated for each animal once daily beginning 1 week prior to initial dosing and throughout the study. "Prima treats" were distributed and the animals' appetite was evaluated approximately 1 hour later.

Ophthalmoscopy: Ocular examinations were conducted once prior to initial dosing, and once at the end of treatment period.

EKG: Multilead ECGs were recorded for all animals once prior to initial dosing, and once at the end of treatment period.

Hematology: Blood samples were collected prior to the initial dosing, at the end of treatment (Day 15) and recovery period (Day 28).

Clinical chemistry: Blood samples were collected prior to the initial dosing, at the end of treatment (Day 15) and recovery period (Day 28).

Urinalysis: Samples were collected prior to the initial dosing, at the end of treatment (Day 15) and recovery period (Day 28).

Gross pathology: Full necropsy was performed on all monkeys at the scheduled termination (Day 15 or Day 28).

Organ weights: See "Histopathology Inventory" table below.

Histopathology: The tissues and organs listed in "Histopathology Inventory" table below were collected from all necropsied animals and placed in 10% neutral-buffered formalin except noted otherwise. Epididymides and testes were fixed in Bouin's solution, and the eyes with optic nerves were fixed in Zenker's solution. The collected tissues/organs were processed and stained with hematoxylin and eosin/phloxine for microscopic examination. Parathyroids and optic nerves were only examined if present in routine sections.

Adequate Battery: Yes

Peer review: No

Toxicokinetics/Immunogenicity: Blood samples were collected at approximately 5, 15, 45, 60, 120, and 360 minutes post dosing of Dose 1 (Day 1) and Dose 14 (Day 14). One set of monkey plasma samples were analyzed for levels of DX-88 using HPLC/MS method (Technical study No. DX005) by (b) (4). Another set of samples were analyzed for anti-DX-88 antibodies using a validated ELISA method developed by (b) (4) (Study No. 18892). A rabbit anti-ecallantide IgG was used as the positive control which was detected by secondary goat-anti-rabbit antibody conjugated to alkaline phosphate.

Results

Mortality: All animals survived to the scheduled necropsy.

Clinical signs: There were no test article-related clinical observations.

Body weights: There were no test article-related effects on body weights or body weight changes.

Food consumption: No report for food consumption. There were no test article-related effects on the semi-quantitative assessment of appetite.

Ophthalmoscopy: There were no test article-related ophthalmic lesions observed.

EKG: There were no test article-related effects noted in ECG evaluation.

Hematology: There were no test article-related alterations of measured parameters.

There were no significant effects on the measured APTT, PT and fibrinogen values.

Clinical Chemistry: There were no significant test article-related adverse effects on the measured parameters. The mean ALT and AST values on Day 15 were elevated in HD males (ALT: 124 IU/L, +39%, AST: 218 IU/L, +114%) and in LD females (ALT: 167 IU/L, +203% , AST 216 IU/L, +254%) when compared to the controls. The differences were attributed to the single high values in one HD male and one LD female of each group. Since the increase in ALT and AST was not associated with any corresponding histopathology findings was not dose-dependent, it was unlikely to be test article-related toxicity. The markedly elevated CK (5876-19690 IU/L) and LDH values (1250-2290 IU/L) were observed in one HD male, one MD male and one LD female, and the moderate elevated CK (> 1075 - 2254 IU/L) or LDH values (500-622 IU/L) were observed in two pre-dose males, two pre-dose females and one HD female (CK only). Since there were no associated clinical signs and histopathology findings, this elevation was more likely to be dosing procedure-related.

Urinalysis: There were no test article-related alterations of measured parameters.

Gross pathology: There were no test article-related gross findings.

Organ weights: There were no test article-related effects on organ weights.

Histopathology: There were no significant test article-related microscopic findings indicative of toxicity of DX-88.

Toxicokinetics: The summary of data was listed below (excerpted from appendix V, Table 7). Plasma DX-88 concentration decreased biphasically with a rapid distribution phase having a half life ($\alpha/2$) of approximately 2 - 7 minutes and a terminal elimination half lift ($\beta/2$) of 40-100 minutes. Both Cmax and AUC_(0-360min) seem to increase in a dose proportional fashion. No significant differences are observed in total body clearance (Cl) between sexes. Volume of distribution at steady state (Vss) is found to be similar in all animals.

Table 7. Mean Pharmacokinetic Parameters normalized to 2.3 mg/kg/day

Day 1

Males			Females	
Dose (mg/kg/day)	Mean Cmax (µg/mL)	Mean AUC _(0-360 min) (µg.min/mL)	Mean Cmax (µg/mL)	Mean AUC _(0-360 min) (µg.min/mL)
2.3	56.72	894.17	46.36	1,077.55
6.8	81.31	926.72	60.65	833.28
23.0	36.19	701.26	60.33	1,228.34

Day 14

Males			Females	
Dose (mg/kg/day)	Mean Cmax (µg/mL)	Mean AUC _(0-360 min) (µg.min/mL)	Mean Cmax (µg/mL)	Mean AUC _(0-360 min) (µg.min/mL)
2.3	29.38	531.88	35.30	1,104.25
6.8	43.94	655.76	52.56	723.82
23.0	24.30	636.16	78.67	921.28

Immunogenicity: There were no significantly high titers found in the monkey plasma (referred appendix VIII, page A-224), indicating no anti-drug antibodies detected in the samples collected.

Histopathology inventory:

Study	(b) 446021	(b) 446020	(b) 446010	(b) 3130	(b) 1574	(b) 446004
Species	Rats 6 months	Monkeys 6 months	Monkeys 90-Day	Monkeys 14-day, iv	Rats 14-day, iv	Rats 14-day, iv
Adrenals	X*	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X
Bone (femur)	X	X	X	X	X	X
Brain	X*	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X	X
Cervix	X	X*	X*	X*		
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymides	X	X	X	X	X	X
Esophagus	X	X	X	X	X	X
Eyes	X	X	X	X	X	X
Fallopian tube						
Gall bladder		X	X	X		
Gross lesions	X	X	X	X	X	X
Harderian gland	X					X
Heart	X*	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X	X
Injection site	X	X	X	X	X	X
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*
Lacrimal gland					X	
Larynx						
Liver	X*	X*	X*	X*	X*	X*
Lungs	X	X	X	X	X*	X
Lymph nodes, cervical						
Lymph nodes mandibular	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X
Nasal cavity						
Optic nerves	X	X	X	X	X	X
Ovaries	X*	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X	X
Parathyroid	X*	X*	X*	X*	X*	X*
Peripheral nerve						
Pharynx						
Pituitary	X*	X*	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*	X*
Rectum	X	X	X	X		X
Salivary gland	X	X	X	X	X	X

Sciatic nerve	X	X	X	X	X	X
Seminal vesicles	X	X*	X*	X	X*	X
Skeletal muscle	X	X	X	X	X	X
Skin (non-injection site)	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X*
Sternum	X	X	X	X	X	X
Stomach	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*	X*	X*
Tongue				X	X	X
Trachea	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X
Uterus	X*	X*	X*	X*	X*	X*
Vagina	X	X	X	X	X	X
Zymbal gland						
Other organ/tissue	Ureters	Ureters	Ureters	Mediastinal Lymph Node		

X, histopathology performed, *, organ weight obtained
 a. In this study, all the checked tissues/organs were collected but not all of them were examined (see the text of this study review above)

2.6.6.4 Genetic toxicology

Not conducted.

2.6.6.5 Carcinogenicity

Not conducted.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Fertility and General Reproduction Toxicity Study of DX-88 Administered Subcutaneously in Rats

Key study findings:

One 25 mg/kg male rat was moribund sacrificed due to the severe injection sites reaction. The test article related paternal and maternal toxicity was limited to the irritation of the injection sites at 10 and 25 mg/kg dose levels. The NOAEL for local toxicity was identified at 5 mg/kg and the NOAEL for systemic toxicity was identified at 25 mg/kg. No test article-related effects were observed on the mating or fertility of the male rats. The male reproductive NOAEL is 25 mg/kg. No test article-related effects were observed on estrous cycle, mating, fertility, and C-sectioning and litter parameters. The female NOAEL for reproductive function and early embryonic development is identified at 25 mg/kg. There were no effects on mating or reproductive performance in rats with subcutaneous doses up to 25 mg/kg.

Study no.: 2204-103

Volume #, and page #: Electronic submission

Conducting laboratory and location: (b) (4)

Date of study initiation: January 27, 2006

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: DX-88 drug product in PBS, pH 7.0, Lot # 227-05-001, purity, 99.58% (product) 94.54% (DX-88)

Methods

Doses: 0, 5, 10, and 25 mg/kg administered daily

Species/strain: Rats/Crl:CD[®](SD) (b) (4)

Age at delivery: approximately 62 days for males and females

Body weight at study assignment: Male – 326~388 g, Female - 222~266 g

Number/sex/group: 25/sex/group

Route, formulation, volume, and infusion rate: Rats were dosed by the subcutaneous route as shown in the table below.

Dosage Group	Dosage* (mg/kg/day)	Nominal Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats Per Sex	Assigned Numbers	
					Male Rats	Female Rats
I	0 (Vehicle)	0	2.5	25	6301 - 6325	6401 - 6425
II	5	10.0	0.5	25	6326 - 6350	6426 - 6450
III	10	10.0	1.0	25	6351 - 6375	6451 - 6475
IV	25	10.0	2.5	25	6376 - 6400	6476 - 6484, 6485, 6486 - 6500

Satellite groups used for toxicokinetics: NA

Study design: Male rats were dosed once daily beginning 28 days before cohabitation, through the cohabitation period (maximum 14 days) and continuing through the day before sacrifice (totals of 48 to 51 daily doses). All surviving male rats were sacrificed on SD 49 or 52. Female rats were dosed once daily beginning 15 days before cohabitation and continuing through day 7 of gestation (total 23 to 36 daily doses). Females were sacrificed on GD 13, and caesarean-sectioned.

The cohabitation period consisted of a maximum of 14 days. Females rats with spermatozoa observed in a smear of the vaginal contents and/or copulatory plug in situ were considered to be GD 0 and assigned to the individual housing. The females were not mated after the completion of the 14-day cohabitation period were considered to be at day 0 of presumed gestation and assigned to the individual housing.

Parameters and endpoints evaluated: Rats were observed for viability twice daily. Clinical signs, abortions, premature deliveries and deaths were checked

daily before dosing, approximately 60 min post dosing, once daily during post-dosage period (female rats), and on the day of sacrifice. These observations were also recorded at approximately 15 min post dosing for all rats on the 1st day of dosing and the seven rats on the 2nd day of dosing.

Body weights were recorded at least weekly during the pre-dosage period, daily during the dosing period and post-dosage period, and on the day of termination. Food consumption values for males were recorded weekly during the dosing period except during cohabitation. Food consumption values for females were recorded weekly to cohabitation and on GDs 0, 5, 8, 10 and 13.

Estrous cycling was evaluated by examination of vaginal cytology for 14 days before initial dosing, for 14 days beginning with the 1st day of post dosing and then until spermatozoa and/or copulatory plug were observed.

For male rats, a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The reproductive organs including right testis, left epididymis (whole and caudal), right epididymis, seminal vesicles, and prostate were individually weighed. Gross lesions, the right epididymis, the remaining left epididymis (corpus and caput), seminal vesicles, prostate and testes were fixed for possible histopathology evaluation. Sperm concentration and motility were evaluated using computer-assisted sperm analysis.

For the unscheduled death male rat, gross necropsy for lesions was performed. The lung, heart, liver, kidneys, stomach, spleen, testes and epididymides were fixed for possible histopathology evaluation.

For female rats, a gross necropsy of the thoracic, abdominal and pelvic viscera was performed and pregnancy status was checked. Gross lesions, ovaries and uterus of each female rat were fixed for possible histopathology evaluation. The number and distribution of corpora lutea, implantation sites, viable and nonviable embryos were recorded and placentae were examined for size, color and shape.

Results

Mortality: One male rat in the 25 mg/kg group was sacrificed due to the severe injection site reactions on SD 37, approximately 4.5 hours post dosing. Clinical signs for this rat included injection sites discoloration (red, purple, green and/or black) on SD 8 through SD 37, scabbing on SD 22 to SD 37, and ulceration on SD 31 to SD 37. Food consumption was reduced on SD 22-28. All other tissues appeared normal at necropsy. One male rat in the 10 mg/kg group was sacrificed due to an injury to the snout on SD 5. This unscheduled death was unlikely to be test article-related.

All other rats survived till the scheduled termination.

Clinical signs: For all surviving rats, test article-related clinical signs were irritation (discoloration and scabbing) at the injection sites of the test article-treated males and females. Discoloration (red, purple, green and/or black) occurred in significantly increased numbers of males at all test article dose levels and scabbing occurred in significantly increased numbers of males at 10 and 25 mg/kg dose levels. Significantly increased numbers of females in 10 and 25 mg/kg dose groups showed discoloration and/or scabs at the injection sites. Additionally, ulceration at the injection sites occurred in 4 males in the 25 mg/kg group, in 1 female in the 10 mg/kg group and 3 females in the

25 mg/kg group during the gestation period. There were no other significant test article-related clinical signs.

Body weight: There were no test article-related effects on body weights and body weight gains in males and females. The increase in body weight gain of male rats in prehabitation period was in small magnitude (up to +15 g or +4% body weight) and not in a dose-dependent pattern, hence, was not considered toxicologically significant though that was statistically significant. Average body weights were comparable among control and all treated males. Body weight gain for females during prehabitation period (SD 1-15) was increased in a dose-independent manner in all test article treated animals (up to +7.4 g, +3%) when compared to the control. The body weights over the prehabitation period were comparable among the control and treated females. There was no significant difference in body weight or body weight gain between control and the test article treated females during the gestation period.

Food consumption: There were no significant test article-related changes on food consumption. The increased food consumption in isolated two intervals with inconsistent dosage levels was not considered toxicologically significant.

Toxicokinetics: Not measured.

Necropsy: There were no significant macroscopic findings indicating test article-related toxicity. The macroscopic findings for the unscheduled death males were described in the section of mortality. The finding of a firm yellow mass on the left cauda epididymis was not considered test article-related since it was only observed in one 25 mg/kg male rat and this rat (No. 6394) impregnated the cohort female rat. .

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): The data were summarized in the tables below (Tables C10-page 1 and C10-page 2, and C12 excerpted from pages 113, 114 and 116).

TABLE C10 (PAGE 1): ESTROUS CYCLING AND MATING AND FERTILITY - FEMALE RATS

DOSAGE GROUP	I	II	III	IV
DOSAGE (MG/KG/DAY) ^a	0 (VEHICLE)	5	10	25
ESTROUS CYCLING OBSERVATIONS				
RATS EVALUATED	25	25	25	25
PREDOSSAGE ESTROUS CYCLING				
ESTROUS STAGES/ 12 DAYS	MEANS ± SD	3.5 ± 0.5	3.4 ± 0.8	3.3 ± 0.6
RATS WITH 4 OR MORE CONSECUTIVE DAYS OF DIESTRUS	0	0	0	0
RATS WITH 3 OR MORE CONSECUTIVE DAYS OF ESTRUS	0	0	0	0
PRECONCEPTION ESTROUS CYCLING				
ESTROUS STAGES/ 10 DAYS	MEANS ± SD	3.3 ± 0.4	3.2 ± 0.3	3.2 ± 0.3
RATS WITH 4 OR MORE CONSECUTIVE DAYS OF DIESTRUS	1	0	0	0
RATS WITH 3 OR MORE CONSECUTIVE DAYS OF ESTRUS	0	0	0	0

a. Dosage occurred on day 1 of study through day 7 of predated gestation.

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TABLE C10 (PAGE 2): ESTROUS CYCLING AND MATING AND FERTILITY - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 25
MATING OBSERVATIONS					
RATS IN COHABITATION	N	25	25	25	25
RATS IN COHABITATION b	MEANS ± S.D.	3.3 ± 3.8	3.1 ± 2.2	2.3 ± 1.3	2.2 ± 1.8
RATS THAT MATED	N(N)	24(96.0)	24(100.0)	25(100.0)	24(100.0)
FERTILITY INDEX c	N/N	24/24	24/24	25/25	24/24
	(%)	(100.0)	(100.0)	(100.0)	(100.0)
RATS WITH CONCEIVED LITTERS d	N	24	25	25	25
LITTERS BY DAMS e	N(N)	22(91.7)	24(96.0)	25(100.0)	24(96.0)
LITTERS f-g	N(N)	21(87.5)	16(64.0)	21(84.0)	21(84.0)
RATS PREGNANT/DAMS IN COHABITATION	N/N	24/24	24/25	25/25	24/25
	(%)	(100.0)	(96.0)	(100.0)	(100.0)

a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Mating occurred on day 1 of study through day 7 of gestation.
c. Number of estrous stages per 14 days.
d. Restricted to rats with a confirmed mating date.

PROTOCOL 2204-003: FERTILITY AND GENERAL REPRODUCTION TOXICITY STUDY OF DX-98 ADMINISTERED SUBCUTANEOUSLY IN RATS

TABLE C12 (PAGE 1): CAESAREAN-SECTIONING AND LITTER OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 25
RATS TESTED	N	25	25	25	25
PREGNANT	N(N)	24(96.0)	24(96.0)	24(96.0)	25(100.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION					
	N	24	24	24	25
CORPORA LUTEA	MEANS ± S.D.	15.8 ± 2.1	15.7 ± 2.4	16.3 ± 1.9	15.4 ± 1.8
IMPLANTATIONS	MEANS ± S.D.	16.2 ± 1.7	15.2 ± 2.1	15.8 ± 1.5	15.0 ± 1.6
VARIABLE EMBRYOS	N	344	340	338	338
	MEANS ± S.D.	14.3 ± 2.2	14.2 ± 2.3	14.1 ± 3.2	13.5 ± 2.2
NONVIABLE EMBRYOS	N	21	25	40	37
	MEANS ± S.D.	0.9 ± 3.0	1.0 ± 1.5	1.7 ± 2.2	1.5 ± 1.3
DAMS WITH ALL NONVIABLE EMBRYOS	N(N)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(N)	24(100.0)	24(100.0)	24(100.0)	25(100.0)
PLACENTAE APPEARED NORMAL	N(N)	24(100.0)	24(100.0)	23(95.8)	25(100.0)
% NONVIABLE CONCEPTUSES/LITTER	MEANS ± S.D.	6.0 ± 7.6	6.6 ± 9.7	11.4 ± 15.8	10.1 ± 8.9

a. Dosage occurred on day 1 of study through day 7 of gestation.

There were no test article-related effects on estrous cycle, mating or fertility observed. The number of estrous stages per 14 days was comparable among all groups. All mating and fertility parameters were comparable among four groups. There were no significant test article-related effects observed on the C-sectioning or litter parameters. The litter averages for corpora lutea, implantations, viable and nonviable embryos and percent nonviable embryos per litter were comparable among the four dose groups. No dam had a litter consisting of only nonviable embryos.

There were no test article-related effects on the weights of the selected reproductive organs.

Sperm analysis data were summarized in the tables below (Tables B10 and B11 excerpted from pages 49 and 50). In a weak dose-dependent pattern, the reduction of cauda epididymal sperm count was -13%, -16% and -18%, and the reduction of sperm

density was -10%, -13% and -16%, at doses of 5, 10 and 25 mg/kg, respectively. Considering the small magnitude of the reduction and the variation of the values, these changes were unlikely to be a test article-related adverse effect on sperm. The values for number and percent motile sperm, number of nonmotile sperm and total sperm count from the vas deferens were comparable among four groups. There was no test article-related change of sperm morphology.

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TABLE B10 (PAGE 1): SPERM MOTILITY, COUNT AND DENSITY - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	10	25
RATS TESTED	N	25	25	24a	24b
VAS DEFERENS SPERM MOTILITY					
NUMBER MOTILE	MEANS ± S.D.	330.0 ± 148.0	335.0 ± 105.5 (.24)c	312.3 ± 89.2	281.0 ± 78.6 (.23)d
MOTILE PERCENT	MEANS ± S.D.	91.4 ± 5.7	90.6 ± 11 (.24)c	89.3 ± 11.1	90.5 ± 6.0 (.23)d
STATIC COUNT (NONMOTILE)	MEANS ± S.D.	28.5 ± 18.4	32.9 ± 35.5 (.24)c	36.1 ± 34.6	23.1 ± 17.4 (.23)d
TOTAL COUNT	MEANS ± S.D.	358.5 ± 152.8	368.7 ± 101.9 (.24)c	348.4 ± 86.8	304.0 ± 78.7 (.23)d
CAUDA EPIDIDYMAL SPERM COUNT					
SPERM COUNT	MEANS ± S.D.	134.1 ± 34.7	116.0 ± 52.2 (.24)c	112.1 ± 18.0*	104.9 ± 17.6** (.23)d
SPERM DENSITY	MEANS ± S.D.	1375.18 ± 327.96	1053.90 ± 261.61 (.24)c	1024.27 ± 187.07*	984.25 ± 107.13** (.23)d

TABLE B11 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	10	25
RATS EXAMINED	N	25	25	24a	24b
INCLUDED IN ANALYSES	N	25	24	24	23
NORMS	MEANS ± S.D.	104.9 ± 5.6	104.2 ± 5.8	105.0 ± 7.3	102.1 ± 4.5
DETACHED HEAD	MEANS ± S.D.	5.2 ± 1.4	6.3 ± 3.4	7.1 ± 4.8	6.6 ± 4.1
NO HEAD	MEANS ± S.D.	2.4 ± 2.2	1.3 ± 1.8	1.5 ± 2.8	2.1 ± 1.6
BROKEN FLAGELLUM	MEANS ± S.D.	0.2 ± 0.3	0.5 ± 0.8	0.2 ± 0.5	0.2 ± 0.5
COILED FLAGELLUM	MEANS ± S.D.	0.1 ± 0.3	0.0 ± 0.0	0.2 ± 0.5	0.0 ± 0.2
BENT FLAGELLUM	MEANS ± S.D.	0.2 ± 0.4	0.0 ± 0.0	0.1 ± 0.3	0.2 ± 0.3
BENT EPIDIDYMAL TIP	MEANS ± S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PERCENT NORMAL	MEANS ± S.D.	92.4 ± 2.4	92.5 ± 3.3	92.5 ± 3.8	92.7 ± 2.4

Embryofetal development

Study title: Intravenous Development Toxicity Study of DX-88 in Pregnant Rats

Key study findings:

Maternal toxicity:

There was one early death in the 20 mg/kg group post the 6th daily dose. Adverse clinical observations included decreased motor activity in the 15 and 20 mg/kg dose groups and more clinical observations in the 20 mg/kg group. Maternal body weight gains were reduced during the dosing period for the 15 and 20 mg/kg groups (i.e., 56.3 and 38.8 g, respectively, versus 76.9 g for the control). Thus, there was evidence of significant maternal toxicity at 20 mg/kg; however,

the maternal toxicity at 15 mg/kg was judged to be relatively mild. The NOAEL was identified at 10 mg/kg.

Development toxicity:

Increased the number of early resorptions and the percentage of resorbed conceptuses per litter with corresponding reduced number of live fetuses, reduced fetal body weights (-11%), increased incidence of fetal alterations, including marked dilation of the lateral ventricle of the brain, short tail, vertebrate/rib malformations in the 20 mg/kg group. These findings occurred in the presence of significant maternal toxicity.

Increased the number of early resorptions and the percentage of resorbed conceptuses per litter without significant reduced number of live fetuses and reduced fetal body weights (-4%) in the 15 mg/kg group. These findings occurred in the presence of relatively mild maternal toxicity and should be reported in the labeling.

The development NOAEL was identified at 10 mg/kg (AUCinf=7.55 ug.hr/mL= 453 ug.min/mL after single dose of 10 mg/kg).

Study no.: (b) L 2204-001

Volume #, and page #: Electronic submission

Conducting laboratory and location:

(b) (4)

Date of study initiation: June 8, 2004

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: DX-88, Lot # 04RP005, purity: 94.5% (99.5% for product).

Methods

Doses: 0, 10, 15 and 20 mg/kg from day 7 through 17 of gestation for day. See study design tables below (excerpted from page 17 of the report).

Dosage Group	Dosage* (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats per Sex	Assigned Rat Numbers
I	0 (Vehicle)	0	2.0	25	11501 - 11525
II	10	10.0	1.0	25	11526 - 11550
III	15	10.0	1.5	25	11551 - 11572, 5146 ^b , 11574, 11575
IV	20	10.0	2.0	25	11576 - 11600

Species/strain: Presumed pregnant female Sprague-Dawley, outbred albino rats /CrL:CD®(SD)IGS BR VAF/Plus® (b) (4) Virgin female rats were mated with males (one male/female) and rats observed with spermatozoa in vaginal smears or copulatory plugs *in situ* were considered to be at day 0 of gestation, (GD 0).

Body weight: 217-252 g at study assignment

Age: 62 days at arrival

Number/sex/group: 25/females/group

Route, formulation, volume, and infusion rate: IV administration, vehicle was PBS. See the study design tables above.

Satellite groups used for toxicokinetics: None. (note: the TK parameters were evaluated in the dose-range finding study described in the dose selection below)
Study design: Presumed-pregnant rats were administered intravenously once daily with vehicle control or DX-88 at dose levels of 0, 10, 15 and 20 mg/kg on days 7 through 17 of gestation. On day 21 of gestation, all surviving female rats were sacrificed for Cesarean-sectioning and a gross necropsy.

Dose selection: Dose selection was based on a rat dose range-finding study (b) (4) 2204-001P, EDR, section 4.2.3.5.2 titled "*Intravenous Dosage-Range Development Toxicity Study of DX-88 in Rats*". In this study, 8 presumed pregnant female rats/group were intravenously administered with vehicle control or DX-88 (lot No. 04RP005, liquid form in PBS, pH 7.4) at dose levels of 5, 10, 25 and 50 mg/kg/day from day 7 of presumed gestation (GD 7) through day 17. An additional 15 presumed pregnant rats/group were treated once at doses of 0 (vehicle), 5, 10, 25 and 50 mg/kg on GD 7 for toxicokinetic and APTT evaluation. Blood samples for TK and APTT evaluation were collected approximately 0.25, 0.5, 1 and 4 hours postdose on GD 7. A validated HPLC/MS method (Technical study No. AA16809-UXZ) was used to determine plasma concentration of DX-88 in TK analysis. All surviving main study rats were sacrificed on GD 21 for gross necropsy and Cesarean-sectioning. Fetuses were examined for external alterations and sex.

Three main study rats in the 50 mg/kg group and two rats in the 25 mg/kg groups (one in main study and one in TK group) were found dead on GDs 7 or 9. No significant clinical observations were reported for the found dead rats in the 25 mg/kg and one 50 mg/kg before death. Adverse clinical observations for the two 50 mg/kg found dead rats before death included decreased motor activity and/or ptosis, lacrimation and increased depth of respiration on GDs 7 and/or 8. There were no significant necropsy findings in these found dead rats. The litters consisted of 13, 14 or 15 embryos.

All other rats survived to the scheduled termination. Observations including decreased motor activity, red or brown perivaginal substance, excess salivation, increased depth of respiration and urine-stained abdominal fur were found with higher incidence in the rats of the 25 and 50 mg/kg groups. One rat in the 50 mg/kg showed lost righting reflex, prostrate, tremors, ptosis, lacrimation and tachypnea. Body weight gains in the entire dosing period (GD 7-18) were slightly reduced in the 25 (+32 g vs. +66.8 g in the control) and 50 mg/kg groups (+13.4 g vs. +66.8 in the control). Mean body weight in the 25 and 50 mg/kg groups were 84% and 78% of the control group value on GD 21. Relative food consumption values (in g/kg/day) were slightly reduced up to 14% in the 50 mg/kg group during dosing period but increased over the control group values during the postdosing period. APTT values were increased in a dose-dependent manner in all test article-treated groups at time points up to 1 hour post dose. At 4 hour post dosing, APTT remained elevated in the 50 mg/kg group while the APTT values of the other test article-treated groups were comparable to the control. No test article-related gross findings were observed. Pregnancy occurred in 7 (87.5%), 6(75.0%), 6(75.0%), 7 (87.5%) and 7 (87.5%) rats in the control, 5, 10, 25 and 50

mg/kg groups, respectively. The numbers of early resorptions were increased in the 25 and 50 mg/kg groups. Consequently, mean litter size and the number of live fetuses were reduced, and the percent resorbed conceptuses per litter was markedly increased in these two groups. Fetal body weights (grams/litter) were reduced by 9% and 30% in the 25 and 50 mg/kg groups, respectively, when compared to the control. Gross examinations found no external fetal abnormalities. Based on these results, dose of 0, 10, 15 and 20 mg/kg selected for definitive development study were considered reasonable.

TK parameters were summarized in the section of TK below.

Parameters and endpoints evaluated: The dams were evaluated for survival (twice/day), clinical observations, abortion and premature delivery and deaths (prior to and at approximately 15 and 60 minutes post dose in dosing period and once daily during the postdosing period), body weight (on GD 0, and daily through the study) and food consumption (on GDs 0, 7, 10, 12, 15, 18 and 21). On GD 21, rats were sacrificed, Cesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of corpora lutea, implantation sites, early and late resorptions, live and dead fetuses were recorded and placentae were examined for size, color and shape. Fetuses were individually weighed, and evaluated for gender, body weight, gross external alterations and visceral or skeletal alterations.

Results

Mortality (dams): One female rat was found dead on GD 12 about 3 hours post the 6th daily dose of 20 mg/kg. The clinical observations of this rat before death included decreased motor activity on GDs 7 and 9, and ptosis on GD 7. No significant body weight gain and food consumption changes were observed. No significant findings were observed at necropsy. The rat was not pregnant. Other rats survived to scheduled termination.

Clinical signs (dams): Clinical observations with higher incidences in the test article-treated groups than in the control group included decreased motor activity, urine-stained abdominal fur observed in the 15 and 20 mg/kg dose groups, and ptosis, excess salivation, red perivaginal substance and red perioral substance, pale extremities, soft/liquid feces, chromdacroryrria, lost righting reflex and tachypnea observed in the 20 mg/kg rats. The other clinical observations were not considered test article-related.

Body weight (dams): As shown in the table below, body weight gain in the test article-treated groups was reduced in a dose-dependent pattern during the dosing period (GDs 7-18) and the gestation period (GDs 7-21). The reductions of the body weight gains in the gestation period were -9% and -22% in the 15 and 20 mg/kg groups, respectively. Maternal body weights were reduced in the 15 mg/kg group up to -8% and in the 20 mg/kg group up to -16% at the end of dosing and gestation periods.

Dose (mg/kg)	0	10	15	20
No. of tested rats	25	25	25	25
No. of pregnant rats	22	24	24	24

Body weight Gain (g) during GDs 7-18	76.9	65.7	56.3	38.9
% Body weight Gain (%), GDs 7-18	27.9	24.0	20.8	14.2
% Body weight of the control value on GD 18	100.0	96.0	92.5	88.8

Note: The reduction of body weight gain or body weight = % body weight gain or % body weight in the treated group - % body weight gain or % body weight in the control

Food consumption (dams): Absolute (g/day) and relative (g/kg/day) food consumption values were slightly reduced in all treated groups up to -11 % in the 20 mg/kg dose group, during the entire dosing period (GDs 7-18).

Toxicokinetics: No TK evaluation was performed for this definitive study. However, TK parameter was evaluated in the DRF study (No. (b)2204-001P). Values are listed in the table below (excerpted from Study (b) 2204-001P, Page 12 of the study report).

Table from Dose Range Finding Study

Group	Dosage (mg/kg)	C _{max} (mcg/mL)	t _{max} (h)	t _{last} (h)	AUC _{last} (mcg·h/mL)	AUC (mcg·h/mL)	t _{1/2} (h)	V _z (mL/kg)	CL (mL/h·kg)
II	5	4.76	0.25	0.5	1.49	NE	NE	NE	NE
III	10	13.5	0.25	1	6.65	7.55	0.29	545	1320
IV	25	33.9	0.25	1	17.8	21.8	0.35	587	1150
V	50	145	0.25	1	73.5	84.0	0.29	252	595

NE: Not estimated, due to insufficient characterization of the terminal phase.

Terminal and necropsic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

No drug-related gross lesions were observed at maternal necropsy. The right horn of the uterus was reduced to a ligament in one dam of the 10 mg/kg group, which had a unilateral pregnancy in the left uterine horn. Since this was the isolated finding in one rat, it was unlikely to be test article-related.

The C-sectioning and litter observation data was summarized in the tables below (excerpted from page B-8 of the report). Two rats in the control group and one rat in the 15 mg/kg group delivered a litter before C-sectioning on GD 21. C-section observations were based on 20, 24, 23 and 24 pregnant rats with ≥ 1 live fetuses in the 0, 5, 15 and 20 mg/kg dose groups, respectively. As shown in the tables below, number of early/total resorptions and percentage of resorbed conceptuses per litter were increased in the 15 and 20 mg/kg dose groups, when compared to the control values. The corresponding reduced litter size/number of live fetuses per litter and reduced fetal body weight (-11%) were significant in the 20 mg/kg group but insignificant in the 15 mg/kg group. Two dams in the 20 mg/kg had all conceptuses resorbed. For the 20 mg/kg group, due to the maternal toxicity including adverse clinical observations, significantly decreased body weight gain, and one death, the test article-related findings in C-sectioning and litter observations may be secondary to the maternal toxicity. However maternal toxicity was judged to be mild for the 15 mg/kg group and increased numbers of early/total resorptions and percentages of resorbed conceptuses per litter might be attributed to DX-88.

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DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 10	III 15	IV 20
RATS TESTED		N	25	25	25
PREGNANT		N(%)	22 (88.0)	24 (96.0)	24 (96.0)
DELIVERED AND SACRIFICED		N(%)	2 (9.1)	0 (0.0)	1 (4.2)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION		N	20	24	23
CORPORA LUTEA	MEAN±S.D.	15.7 ± 1.1	15.5 ± 2.9	16.5 ± 2.7	16.5 ± 2.9
IMPLANTATIONS	MEAN±S.D.	14.8 ± 1.6	13.8 ± 3.1	15.1 ± 1.7	15.1 ± 2.3
LITTER SIZES	MEAN±S.D.	14.2 ± 1.5	13.0 ± 3.4	11.2 ± 5.0	6.0 ± 4.4**
LIVE FETUSES	N	284	311	258	143
	MEAN±S.D.	14.2 ± 1.5	13.0 ± 3.4	11.2 ± 5.0	6.0 ± 4.4**
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.6 ± 0.9	0.8 ± 0.9	3.9 ± 4.1*	9.1 ± 4.0**
EARLY RESORPTIONS	N	12	18	89	216
	MEAN±S.D.	0.6 ± 0.8	0.8 ± 0.9	3.9 ± 4.1**	9.0 ± 4.0**
LATE RESORPTIONS	N	1	1	0	3
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.0	0.1 ± 0.3
DAMS WITH ANY RESORPTIONS	N(%)	9 (45.0)	12 (50.0)	17 (73.9)	24 (100.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0 (0.0)	1 (4.2)	0 (0.0)	2 (8.3)
DAMS WITH VIABLE FETUSES	N(%)	20 (100.0)	23 (95.8)	23 (100.0)	22 (91.7)
PLACENTAE APPEARED NORMAL ^b	N(%)	20 (100.0)	23 (100.0)	23 (100.0)	22 (100.0)

- a. Dosage occurred on days 7 through 17 of gestation.
- b. Excludes dams with all early resorptions.
- * Significantly different from the vehicle control group value (p≤0.05).
- ** Significantly different from the vehicle control group value (p≤0.01).

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 10	III 15	IV 20
LITTERS WITH ONE OR MORE LIVE FETUSES		N	20	23	22
IMPLANTATIONS	MEAN±S.D.	14.8 ± 1.6	14.3 ± 1.8	15.1 ± 1.7	15.2 ± 2.4
LIVE FETUSES	N	284	311	258	143
	MEAN±S.D.	14.2 ± 1.5	13.5 ± 2.1	11.2 ± 5.0	6.5 ± 4.2**
LIVE MALE FETUSES	N	137	157	130	64
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	48.3 ± 11.0	50.5 ± 12.7	52.2 ± 22.7	39.3 ± 30.4
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.62 ± 0.30	5.57 ± 0.33	5.38 ± 0.41	4.98 ± 0.69**
MALE FETUSES	MEAN±S.D.	5.77 ± 0.31	5.71 ± 0.32	5.55 ± 0.37 [22]b	5.28 ± 0.65** [18]d
FEMALE FETUSES	MEAN±S.D.	5.46 ± 0.29	5.44 ± 0.35	5.19 ± 0.44 [21]c	4.84 ± 0.81* [21]e
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.2 ± 5.7	5.3 ± 6.8	27.3 ± 29.5*	57.6 ± 26.3**

- [] = NUMBER OF VALUES AVERAGED
- a. Dosage occurred on days 7 through 17 of gestation.
- b. Litter 11568 had no male fetuses.
- c. Litters 11554 and 11574 had no female fetuses.
- d. Litters 11579, 11590, 11593 and 11598 had no male fetuses.
- e. Litter 11582 had no female fetuses.
- * Significantly different from the vehicle control group value (p≤0.05).
- ** Significantly different from the vehicle control group value (p≤0.01).

There were no other test article-related adverse effects on C-sectioning or litter parameters. One dam in the 10 mg/kg group had all conceptuses resorbed. It was not considered biologically significant as the single finding was not observed in the next higher dose level of 15 mg/kg. Percent live male fetuses were comparable among control and treated groups. There were no dead fetuses. All placentae appeared normal.

Offspring (malformations, variations, etc.): The number of fetuses with any alteration observed was listed in the table below. Test article-related fetal malformations and/or

variations occurred in the 20 mg/kg dose group, in which the maternal toxicity including mortality, adverse clinical observations and decreased body weight gain were observed.

External and Visceral malformations and variations:

The external alterations included short tail observed in one 20 mg/kg fetus and short trunk observed in another 20 mg/kg fetus. The visceral alterations included marked dilation of the lateral ventricles of brain in two fetuses. One of these fetuses also showed short tail as described above.

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DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0 (VEHICLE)	II 10	III 15	IV 20
LITTERS EVALUATED	N	20	23	23	22
FETUSES EVALUATED	N	284	311	258	143
LIVE	N	284	311	258	143
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	3(15.0)	2(8.7)	7(30.4)	10(45.4)**
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	3(1.0)	2(0.6)	7(2.7)	19(13.3)**
% FETUSES WITH ANY ALTERATION/LITTER	MEANS ± S.D.	1.0 ± 2.4	0.6 ± 2.2	4.4 ± 10.7	19.7 ± 30.6*

a. Dosage occurred on days 7 through 17 of gestation.
* Significantly different from the vehicle control group value (p<0.05).
** Significantly different from the vehicle control group value (p<0.01).

Skeletal malformations and variations: The major findings were listed in the table below (excerpted from pages B-13 to B-16 of the report).

Interrelated vertebrae/rib malformations were observed in the 3 fetuses of 20 mg/kg group which included fused arches and fused right ribs observed in all three fetuses, a hemivertebra present (as the 9th thoracic vertebra or 5th lumbar vertebra) observed in two of these 3 fetuses, and not ossified thoracic vertebrae, fused arches of the 3rd and 4th lumbar vertebrae and fusion of the centra of the sacral and caudal vertebrae observed in one of these 3 fetuses.

Increased incidence of skeletal variations were also observed in the 20 mg/kg fetuses, which included bifid centrum of the thoracic vertebrae, unilateral ossification of the centrum of thoracic or the lumbar vertebrae, small archs of thoracic and lumbar vertebrae, cervical rib present at 7th cervical vertebrae, wavy ribs, and incompletely ossified sternal centra and asymmetric sternal centra, and bent ischia. The increased incidence of these variations was not within the historical control range (refer to the Appendix 7 for rat historical control data in the report of Study ^{(b) (4)} 100012 in EDR section 4.2.3.5.2) and hence, was considered a development toxicity which may attribute to test article-induced maternal toxicity. The other skeletal findings were either comparable to the control or within the historical control range.

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DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0 (VEHICLE)	II 10	III 15	IV 20
LITTERS EVALUATED	N	20	23	23	22
FETUSES EVALUATED	N	146	161	134	79
LIVE	N	146	161	134	79
THORACIC VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.3)	2(8.7)	5(22.7)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)c	2(1.5)	6(7.6)**e, f, g, i
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	4(18.2)**
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	5(6.3)**f, g, h, i

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THORACIC VERTEBRAE: ARCHES, FUSED						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	2(9.1)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	2(2.5)**b,i	
THORACIC VERTEBRAE: HEMIVERTEBRA						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)g	
THORACIC VERTEBRAE: ARCH, SMALL						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)i	
THORACIC VERTEBRAE: CENTRUM, NOT OSSIFIED						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)i	
LUMBAR VERTEBRAE: CENTRUM, BIFID						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)e	
LUMBAR VERTEBRAE: ARCH, SMALL						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)f	
LUMBAR VERTEBRAE: HEMIVERTEBRA						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)i	
LUMBAR VERTEBRAE: ARCHES, FUSED						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)i	

LUMBAR VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(2)	0(0.0)	0(0.0)	0(0.0)	1(1.3)j	
SACRAL VERTEBRAE: CENTRA, FUSED						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)j	
CAUDAL VERTEBRAE: FUSED						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)i	
RIBS: NAVY						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	2(9.1)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	5(6.3)**	
RIBS: FUSED						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	2(9.1)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	3(3.8)**g,h,i	
STERNAL CENTRA: INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(4)	1(5.0)	1(4.3)	3(13.0)	3(13.6)	
FETAL INCIDENCE	N(3)	1(0.7)b	1(0.6)	3(2.2)d	3(3.8)f,g,i	
PELVIS: ISCHIUM, BENT						
LITTER INCIDENCE	N(4)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	
FETAL INCIDENCE	N(3)	0(0.0)	0(0.0)	0(0.0)	1(1.3)i	

Study title: Subcutaneous Developmental Toxicity Study of DX-88 in Rats

Key study findings:

Maternal toxicity:

Injection site irritations including discoloration and scabbing at the injection sites were observed in all test article-treated groups and ulcerations at the injection sites were observed in the 10 and 20 mg/kg groups.

Development toxicity:

No test article-related effects on C-sectioning and litter observations, fetal body weight, sex, and fetal external, visceral and skeletal examinations up to the highest dose tested (20 mg/kg, AUCinf=855483 ng.min/mL). DX-88 was not teratogenic with subcutaneous doses up to 20 mg/kg.

Study no. BEST no. (b) (4) 0012

Volume #, and page #: EDR

Conducting laboratory and location:

(b) (4)

(b) (4)

Date of study initiation: April 5, 2006**GLP compliance:** Yes**QA reports:** yes (X) no ()**Drug, lot #, and % purity:** DX-88 in PBS, pH 7, Lot No. 227-05-001 purity: 99.58% (product) 94.54% (DX-88)**Methods**

Doses: 0, 5, 10 and 20 mg/kg from days 7 through 17 of gestation. See study design tables below (excerpted from page 19 of the report).

Dosage Group	Dosage (mg/kg/day)	Nominal Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned Rat Numbers	
					Main Study	Toxicokinetic Study
I	0 (Vehicle)	0	2.0	25	14301 - 14325	N/A
II	5	10.0	0.5	25 + 8*	14326 - 14350	8826 - 8833
III	10	10.0	1.0	25 + 8*	14351 - 14375	8834 - 8841
IV	20	10.0	2.0	25 + 8*	14376 - 14400	8842 - 8849

The test article was considered 100% active for the purpose of dosage calculations.

N/A - Not Applicable.

a. Eight rats assigned to toxicokinetic sample collection.

Species/strain: Presumed pregnant female Sprague-Dawley, outbred albino rats /CrL:CD®(SD)IGS BR VAF/Plus®, (b) (4) Untreated male and female rats were mated for 5 days. Females with spermatozoa observed in a vaginal smear and/or the presence of a copulatory plug were identified and considered as gestation day 0 (GD 0).

Number/sex/group: 25 female rats/group

Route, formulation, volume, and infusion rate: SC, formulated in phosphate buffered saline; See the study design table above.

Satellite groups used for toxicokinetics: 8 female rats/test article-treated group

Study design: Presumed-pregnant rats was administered subcutaneously once daily with vehicle control or DX-88 at dose levels of 0, 10, 15 and 20 mg/kg on days 7 through 17 of gestation. Injection sites on the back were rotated at 6 different sites to minimize irritation. On day 21 of gestation, all surviving female rats were sacrificed for Cesarean-sectioning and a gross necropsy.

Dose selection: Dose selection was based on iv. rat development toxicity study in rats (Study No. 2204-001) and sc. rat peri/postnatal development toxicity study (Study No. (b) (4) 00006). In Study 2204-001, significant maternal toxicity including one early death was observed at 20 mg/kg; however, the maternal toxicity at 15 mg/kg was judged to be relatively mild. It should be noted that results obtained from intravenous teratology study may not provide a sufficient rationale for the dose selection in the current subcutaneous study. In Study (b) (4) 00006, one 25 mg/kg F0 rat was sacrificed early due to severe ulceration at the injection site and test article-related injection site reactions were observed with increased severity/incidence at the higher doses. Injection site reactions might be a sufficient rationale for limiting the highest subcutaneous dose to 20 mg/kg. Further, since the dose of 25 mg/kg provided 21-fold higher than the

MRHD (2X30 mg s.c) on a mg/kg basis, the high dose selection in the study was considered adequate as a biologic product, for which a high dose with 10-fold of the maximum clinical dose would be acceptable if no target organ of toxicity was identified.

Given the results above and the consideration of a biologic product, the high dose of 20 mg/kg (17-fold of the maximum clinical dose based on mg/kg for a 50 kg person) selected in the current s.c. study was considered acceptable even though maternal toxicity was limited to the local injection site reactions.

Parameters and endpoints evaluated: The dams were evaluated for survival (twice/day), clinical observations, abortion and premature deliveries and deaths (prior to and approximately 60 minutes post dose in dosing period and once daily during the postdosing period), body weight (on GD 0, and daily through the study) and food consumption (on GDs 0, 7, 10, 12, 15, 18 and 21). On GD 7, blood samples were collected from the main study animals at 30 mins and 1, 2, 4, 8 and 16 h post-dose for APTT evaluation and blood samples were collected from TK animals at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h and 24 h post-dose for TK analysis using a validated ELISA method (Technical report No. 06GSTR062). On GD 21, rats were sacrificed, Cesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of corpora lutea, implantation sites, early and late resorption, live and dead fetus were recorded and placentae were examined for size, color and shape. Fetuses were individually weighed, and evaluated for gender, body weight, gross external alterations and visceral or skeletal alterations. (Half of the fetuses were in each litter were examined for soft tissue alterations, using a variation of microdissection technique. The remaining fetuses were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations.)

Results

Mortality (dams): All dams survived until scheduled sacrifice.

Clinical signs (dams): Injection site irritation was observed in all DX-88 treated dams. Irritation was associated with significant discoloration with red, purple, green or black and scabbing at the injection site. One dam in the 10 mg/kg/day group and 2 dams in the 20 mg/kg/day group had ulcerations at the injection sites by the end of the dosing period (GD 17). Onset of irritation and severity of irritation at the injection sites increased with increasing dose.

Body weight (dams): There were no significant differences in maternal body weight/body weight gain between test article-treated groups and the control group.

Food consumption (dams): There were no significant difference in maternal food consumption between test article-treated groups and the control group.

APTT evaluation: Activated partial thromboplastin times (APTT) were longer at time points up to 1 hour post dose in all test article-treated groups (up to 30.83 seconds) and at time points up to 2 hours post dose in the 10 and 20 mg/kg dose groups (up to 32.78

seconds) . However, these increases in APTT were not dose-dependent. At 4 hours post dosing or later time points, the APTT values were generally comparable among the four dose groups.

Toxicokinetics: One TK rat in the 20 mg/kg group was not pregnant. TK parameters are summarized in the table below (excerpted from page 31 of the report). AUC values increased in a dose proportional manner. Volume of distribution values suggested distribution into tissues.

	Group II 5 mg/kg/day	Group III 10 mg/kg/day	Group IV 20 mg/kg/day
$T_{1/2}$ (min)	137.6 ± 33.6	152.0 ± 32.9	158.5 ± 33.7
Cl (mL/min/kg)	22.5 ± 3.55	25.2 ± 7.70	24.2 ± 4.81
Vd (mL/kg)	4423 ± 1210	5659 ± 2392	5440 ± 1054
C_{max} (mcg/mL)	1197 ± 313	1760 ± 740	2477 ± 618
$C_{max}/Dose$ (kg*mcg/mL/mg)	239 ± 62.7	176 ± 74.09*	124 ± 30.9*
T_{max} (min)	37.5 ± 19.6	56.3 ± 29.7	60.0 ± 27.8
AUC _{0-inf} (mcg*min/mL)	227835 ± 39599	424007 ± 105217	855483 ± 167721
AUC _{0-inf} /Dose (mcg*min*kg/mL/mg)	45567 ± 7920	42401 ± 10522	42774 ± 8386

* Statistically different from Group II values at $p < 0.05$ (ANOVA to Student-Newman-Keuls)

Note: the "mcg" in the units of AUC and C_{max} in the table should be "ng" (an error in the text table in the narrative. The original TK report in Appendix 6 was checked).

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

There were no test article-related maternal necropsy findings. One 10 mg/kg rat had numerous pitted areas on the capsule and clear cysts in both kidneys and the liver lobes were rough.

As shown in the tables below (excerpted from page 42-43 of the report), pregnancy occurred in 24/25 rats in each dosage group. One dam in the 5 mg/kg/day group (LD) delivered on GD 21 prior to the scheduled C-section. There were no test article-related effects on Cesarean sections or litter parameters. All measured parameters as listed in the table were comparable between test article-treated groups and the control group.

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DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 20
RATS TESTED		N 25	25	25	25
PREGNANT DELIVERED AND SACRIFICED		N(%) 24 (96.0)	25(100.0) 1(4.0)	25(100.0) 0(0.0)	25(100.0) 0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION		N 24	24	25	25
CORPORA LUTEA	MEAN±S.D.	15.5 ± 1.4	15.8 ± 1.6	15.5 ± 2.1	15.6 ± 2.1
IMPLANTATIONS	MEAN±S.D.	14.6 ± 2.3	15.0 ± 1.6	15.0 ± 1.9	14.6 ± 2.6
LITTER SIZES	MEAN±S.D.	14.2 ± 2.3	14.5 ± 1.8	14.4 ± 2.0	13.8 ± 3.1
LIVE FETUSES	N	340	348	361	344
	MEAN±S.D.	14.2 ± 2.3	14.5 ± 1.8	14.4 ± 2.0	13.8 ± 3.1
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.4 ± 0.8	0.5 ± 0.6	0.6 ± 0.9	0.8 ± 2.4
EARLY RESORPTIONS	N	11	12	15	21
	MEAN±S.D.	0.4 ± 0.8	0.5 ± 0.6	0.6 ± 0.9	0.8 ± 2.4
LATE RESORPTIONS	N	0	0	0	0
DAMS WITH ANY RESORPTIONS	N(%)	8 (33.3)	10(41.7)	10(40.0)	8(32.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	24(100.0)	24(100.0)	25(100.0)	25(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	24(100.0)	25(100.0)	25(100.0)

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 20
LITTERS WITH ONE OR MORE LIVE FETUSES		N 24	24	25	25
IMPLANTATIONS	MEAN±S.D.	14.6 ± 2.3	15.0 ± 1.6	15.0 ± 1.9	14.6 ± 2.6
LIVE FETUSES	N	340	348	361	344
	MEAN±S.D.	14.2 ± 2.3	14.5 ± 1.8	14.4 ± 2.0	13.8 ± 3.1
LIVE MALE FETUSES	N	164	183	193	157
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	47.6 ± 13.1	52.6 ± 13.3	53.3 ± 12.5	44.4 ± 16.2
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.32 ± 0.22	5.40 ± 0.24	5.38 ± 0.27	5.38 ± 0.27
MALE FETUSES	MEAN±S.D.	5.47 ± 0.25	5.55 ± 0.26	5.51 ± 0.28	5.51 ± 0.26 (24) ^b
FEMALE FETUSES	MEAN±S.D.	5.18 ± 0.20	5.25 ± 0.26	5.21 ± 0.26	5.26 ± 0.31
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	3.0 ± 5.0	3.4 ± 4.4	4.0 ± 5.6	5.3 ± 14.3

Offspring (malformations, variations, etc.):

Fetal evaluations were based on live fetuses at the scheduled Cesarean-section delivery on GD 21 and another 14 pups of one dam in the 5 mg/kg group which were delivered on GD 21 prior to the scheduled C-section. There were no test article-related external, visceral, or skeletal malformations or variations.

External and Visceral malformations and variations:

The only external alteration was a protruding tongue observed in one 10 mg/kg fetus. The visceral alterations included fused lateral ventricles of the brain in one 20 mg/kg fetus, microphthalmia and the innominate artery absent in one of each 10 mg/kg fetus, and folded retinas in 1, 2, 1, and 1 fetus from single litter of each dosage group. The external and visceral alterations were not considered test article-related as the findings were either

comparable to the control, not in a dose-dependent pattern or isolated findings to a single fetus.

Skeletal malformations and variations: The skeletal alterations observed were listed in the table below (excerpted from page 47 of the report). As these alterations were either comparable to the control, in the historical control range (Appendix 7 of the report, Sponsor provided historical data), or not in a dose-dependent pattern, they were not considered test article-related.

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 5	III 10	IV 20
LITTERS EVALUATED	N	24	24	25	25
FETUSES EVALUATED	N	175	182	187	178
LIVE	N	175	182	187	178
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA					
LITTER INCIDENCE	N(%)	1 (4.2)	1 (4.2)	2 (8.0)	3 (12.0)
FETAL INCIDENCE	N(%)	1 (0.6)	1 (0.5)	7 (3.7)	4 (2.2)
THORACIC VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	2 (8.3)	0 (0.0)	2 (8.0)	1 (4.0)
FETAL INCIDENCE	N(%)	2 (1.1)	0 (0.0)	2 (1.1)	1 (0.6)
RIBS: SHORT					
LITTER INCIDENCE	N(%)	0 (0.0)	1 (4.2)	2 (8.0)	1 (4.0)
FETAL INCIDENCE	N(%)	0 (0.0)	1 (0.5)	2 (1.1)	1 (0.6)

Study title: Intravenous Development Toxicity Study of DX-88 in Rabbits

Key study findings:

Test article-related mortality was observed at intravenous doses of 5 and 10 mg/kg after the 1st dose. Adverse clinical observations were observed in the 10 mg/kg group. The NOAEL for maternal systemic toxicity was identified at 2 mg/kg. No test article-related embryo-fetal toxicity was observed at the dose levels of 1, 2, 5 or 10 mg/kg (*note: the size of the 10 mg/kg dose group was deficient as it only included 10 pregnant rabbits for evaluation*). Under the conditions of this study, DX-88 was not teratogenic in rabbits up to the tested dose levels of 5 mg/kg/day (AUC_{last}=33.4 µg.hr/mL or 2004 µg.min/mL).

Study no.: (b) (4) 2204-002 and Supplement study No. (b) (4) 0008

Note from the reviewer: Study No. (b) (4) 0008 is a supplement study to Study No. (b) 2204-002. It would be more reasonable to review them together than separately as the supplemental study was to repeat the evaluation in the study (b) 2204-002 at a dose of 2 mg/kg with a larger number of animals. The review below referred to the evaluation of both studies, unless indicated specifically. See details in the study design section for reasons to add the supplemental study.

Volume #, and page #: Electronic submission

Conducting laboratory and location:

(b) (4)

Date of study initiation: September 4, 2004 (supplement study: January 14, 2005)

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: DX-88, Lot # 04RP005, purity: 94.5% (99.5% for product) and Lot# 227-01-004, purity: 94.6% (99.6% for product). Lot#227-01-004 was also used in the supplement study.

Methods

Doses: 0, 1, 5, 10, and 2 mg/kg (for supplement study: doses 0 and 2 mg/kg) from day 7 through 19 of gestation for day. See study design tables below.

Species/strain: Timed-mated female rabbits/Hra:(NZW)SPF, (b) (4)
(b) (4) The day of mating was considered to be GD 0

Number/sex/group: See the study design tables below (excerpted from page 15 of the report and page 14 of the report appendix G for the supplement study).

Study 2204-002

Dosage Group	Dosage ^a (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rabbits	Assigned Rabbit Numbers
I	0 (Vehicle)	0.0	1.0	20	8401 - 8420
II	1	10.0	0.1	20	8421 - 8440
III	5	10.0	0.5	20	8441 - 8460
IV	10	10.0	1.0	10	8461 - 8470
V	2	10.0	0.2	12	8471-8480, 2291, 2292

a. The test article was considered 100% active for the purpose of dosage calculations.

Supplement study (b) (4) 00008

Dosage Group	Dosage ^a (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Injection Rate	Number of Rabbits	Assigned Rabbit Numbers
I	0 (Vehicle)	0.0	0.2	1 minute per injection	20	151-170
II	2	10.0	0.2	1 minute per injection	20	171-173, 9489 ^b , 175-190

a. The test article was considered 100% active/pure for the purpose of dosage calculations.

b. Doe 174 was excluded from study set DG II, due to adverse clinical signs (ungroomed coat and soft and liquid feces) observed before dosage administration and replaced with rabbit 9489.

Route, formulation, volume, and infusion rate: IV administration, vehicle was PBS. See the study design tables above.

Satellite groups used for toxicokinetics: None. (note: the TK parameters were evaluated in the dose-range finding study described in the dose selection below)

Study design: Presumed-pregnant rabbits received DX-88 by intravenous administration at doses of 0, 1, 5 and 10 mg/kg/day from GD 7 through 19. Due to the mortality observed on GDs 7 or 8 (after the 1st dose) in the 5 and 10 mg/kg dose groups, the iv. injection rate was slowed and the last 10 animals originally assigned to the 10 mg/kg group, which had not been dosed, were re-assigned to the additional dosage group of 2 mg/kg along with two extra rabbits (n=12).

(note: the sponsor conducted a supplement study later with n=20 rabbits to confirm the test at 2 mg/kg due to a concern of the number of rabbits less than 16-20/group per ICH Guideline.). All surviving rabbits were sacrificed on GD 29. C-section and gross necropsy of the thoracic, abdominal and pelvic viscera was performed.

Dose selection was based on a rabbit dose range-finding study (b) (4), 2204-002P, EDR, section 4.2.3.5.2 titled "Intravenous Dosage-Range Development Toxicity Study of DX-88 in Rabbits"). In this study, 5 timed-mated female rabbits/group were treated once daily via intravenous administration with vehicle control or DX-88 (lot No. 04RP005, liquid form) at dose levels of 5, 10, 15 and 25

mg/kg/day from day 7 of presumed gestation (GD 7) to day 19. An additional 3 timed-mated rabbits/group were treated once at doses of 0 (vehicle), 5, 10, 15 and 25 mg/kg on GD 7 for toxicokinetic and APTT evaluations.

In toxicokinetic groups, one doe in each of the 10, 15 and 25 mg/kg dose groups were found dead within 15 minutes post dosing on GD 7. No clinical observations before death were observed in the doe of the 10 mg/kg group. Decreased motor activity, ataxia, a clonic convulsion and gasping were observed before deaths of does in the 15 and 25 mg/kg groups; and lost righting reflex, vocalization, miosis of eyes, head tilt to the right and labored breathing were observed in the doe of 25 mg/kg. Gross findings included dark red lining of the trachea and red and dark red or pink, spongy mottled lung lobes observed in all three does and numerous black areas on the thymus observed in the does of 10 and 15 mg/kg groups. The litter sizes ranged from 10 to 12 embryos in each of three does. Due to the severity of the clinical observations before death in the doe of the 25 mg/kg dose group, the remaining two does assigned to this dose group were not dosed (i.e., 25 mg/kg dose group was terminated). In main study animals, two rabbits of the 15 mg/kg group were found dead 7 minutes post dosing on GD 7 (the 1st dosing). Clinical observations before death included decreased motor activity, a clonic convulsion, vocalization, hyperpnea or gasping. No gross findings were observed. The litters consisted of 7 or 9 embryos.

For the remaining animals, test article-related clinical observations included decreased motor activity, clonic convulsions, obsessive grooming, lost righting reflex and tachypnea in the 10 and 15 mg/kg/day dosage groups and hyperpnea, ataxia, vocalization, nystagmus, and gasping in the 15 mg/kg/day dosage group. Clinical signs in the 10 mg/kg group were limited to swollen conjunctiva, hyperreactivity to touch and lacrimation. Body weight gains (GD 7-20) were slightly reduced in the 15 mg/kg group (+0.14 kg vs. control +0.29 kg). Food consumption values were reduced up to 12% in the 10 and 15 mg/kg groups. No test article-related gross findings were observed. Fetal body weights (grams/litter) were reduced about 9% in the 15 mg/kg group when compared to the control. There were no other test article-related effects on Caesarean-sectioning parameters, litter observations and the fetal parameters (sex ratio, external alterations). Based on these result, doses of 0, 1, 5 and 10 mg/kg selected for definitive development study were considered reasonable although some adverse effects had been observed at 10 mg/kg/day.

TK parameters were summarized in the TK section below.

Parameters and endpoints evaluated: The does were evaluated for survival (twice/day), clinical observations, abortion and premature delivery and deaths (prior to and 1 hour post dose in dosing period and once daily during the postdosing period); body weight (on GD 0, the day of arrival and daily through the study) and food consumption (daily from the arrival through the study). On GD 29, rabbits were sacrificed, cesarean-sectioned and gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of corpora lutea, implantation sites, early and late resorption, live and dead fetus were recorded and placentae were examined for size, color and shape.

Fetuses were individually weighed, and evaluated for gender, body weight, gross external alterations and visceral or skeletal alterations.

Results

Mortality (dams): Test article related deaths were observed in the 5 mg/kg and 10 mg/kg dose groups on GDs 7 or 8 after the 1st dosing. Details were described in the table below (excerpted from the page 21 of the report).

Rabbit Number	Mode of Death and Day of Study	Number of Dosages	Clinical Observations	Necropsy Observations
Group III 5 mg/kg/day				
8444	FD DG 8 before 2nd dose	1	No adverse observations.	No adverse observations. Uterine contents: nine embryos.
8448	FD DG 8 before 2nd dose	1	No adverse observations.	No adverse observations. Not pregnant.
Group IV 10 mg/kg/day				
8462	FD DG 7 9 min postdose	1	Decreased motor activity, lost righting reflex, vocalization, red perinasal substance (DG 7)	Lungs: mottled red and dark red; Trachea: contains foamy pink material; all other tissues appeared normal. Uterine contents: nine embryos.
8464	FD DG 8 before 2nd dose	1	Decreased motor activity, lost righting reflex, vocalization, myoclonus, clonic convulsion, splayed limbs, tachypnea, (DG 7)	Stomach: numerous black areas (pinpoint to 0.3 cm x 0.2 cm); all other tissues appeared normal. Uterine contents: nine embryos.
8465	FD DG 7 1 hr, 35 min postdose	1	Decreased motor activity, tachypnea (DG 7)	Lungs: appeared spongy, pale, numerous red areas (pinpoint to 0.2 cm x 0.3 cm); Trachea: numerous dark red areas on mucosal surface (0.3 cm x 0.1 cm to 2.1 cm x 1.0 cm); all other tissues appeared normal. Uterine contents: eight embryos.
8467	FD DG 7 7 min postdose	1	Decreased motor activity, lost righting reflex, clonic convulsion, miosis, pale extremities, gasping (DG 7)	No adverse observations. Uterine contents: nine embryos.

DG = Day of Gestation, FD = Found Dead

In the supplement study, one rabbit in the 2 mg/kg group aborted and was sacrificed on GD 26. Adverse clinical observations before death in this rabbit included soft and liquid feces, purple discoloration of the injection site. Body weight and food consumption were reduced markedly after GD 9. There were no significant necropsy findings. The litter consisted of 8 fetuses. Three fetuses were cannibalized and others appeared normal with external and visceral examination. All fetuses had not ossified pubes at skeletal examination.

All other rabbits survived to the scheduled termination.

Clinical signs (dams): Clinical observations with high incidences in the 10 mg/kg dose group included tachypnea, decreased motor activity, lost righting reflex, splayed limbs and red perinasal substance on GDs 7 and/or 8. Conjunctivitis and/or enlarged third eyelid were observed in a single rabbit of 10 mg/kg group on GDs 8 to 11 and lacrimation was observed in one rabbit each in the 5 and 10 mg/kg groups on GD 8. In supplement study, purple discoloration of the injection sites with higher incidence was observed in the 2 mg/kg rabbits.

No other test article-related clinical signs were observed in both studies.

Body weight (dams): Body weight gains were slightly reduced in the 10 mg/kg group (+0.17 kg vs. control +0.26 kg) during the dosing period (calculated as GDs 7 to 20). Body weight gains were unaffected by doses of DX-88 up to 5 mg/kg (including 2 mg/kg group in the supplement study). Average body weights were comparable in all dose groups.

Food consumption (dams): Absolute food consumption values (g/day) was reduced by 12% in the 10 mg/kg group during the dosing period (calculated as GDs 7-20) when compared to the control, but was comparable to the control during GDs 20- 29. There were no other significant food consumption changes observed.

Toxicokinetics: No TK evaluation was performed for this definitive study. However, TK parameter was evaluated in the DRF study (No. (b) 2204-002P). Values are listed in the table below (excerpted from Study (b) 2204-002P, Page 12 of the study report).

Toxicokinetic parameters from the dose range finding study

Summary of Mean^a DX-88 Plasma Toxicokinetic Parameters

Group	Dosage (mg/kg) ^b	C _{max} (µg/mL)	t _{max} (h)	t _{1/2} (h)	AUC ₀₋₁ (µg·h/mL)	AUC (µg·h/mL)	t _{1/2} (h)	V _d (mL/kg)	CL (mL/h/kg)
II ^c	1	7.75	0.5	4	11.2	11.4	0.66	84.3	89.8
II	5	31.7	0.25	4	33.3	33.4	0.52	112	151
III	10	78.1	0.25	4	134	135	0.58	63.1	74.5
IV	15	95.9	0.25	4	111	113	0.68	136	135

a: Median for t_{max} and t_{1/2}; n=3 at dosages of 1 and 5 mg/kg; n=2 at 10 and 15 mg/kg.

b: Daily dosage.

c: Main study rabbits.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

No gross lesions were revealed in the scheduled maternal necropsy. The gross lesions for 3 found dead rabbits in the 10 mg/kg group and one sacrificed rabbit in the supplement study were described in the section of "Mortality".

The pregnancy rate for the study (b) 2204-002 was listed in the table below (excerpted from page 43 of the report). Due to the mortality in the 5 and 10 mg/kg groups, C-section observations were based on 18, 20, 17, 6 and 12 pregnant does with ≥ 1 live fetuses in the 0, 1, 5, 10 and 2 mg/kg dose groups, respectively. The pregnancy rate was 100% in the supplement study for both control and treated groups. Due to one abortion that occurred in the 2 mg/kg group, the observations were based on 19 pregnant does from the 2 mg/kg group.

For both studies, there were no significant test article-related effects on C-sectioning or litter parameters. No doe had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placetae appeared normal.

TABLE 8 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 1	III 5	IV 10	V 2
RABBITS TESTED	N	20	20	20	10	12
PREGNANT	N(%)	18(90.0)	20(100.0)	18(90.0)	10(100.0)	12(100.0)
FOUND DEAD	N(%)	0(0.0)	0(0.0)	1(5.6)	4(40.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	18	20	17	6	12
CORPORA LUTEA	MEAN±S.D.	10.6 ± 1.6	9.9 ± 2.1	10.0 ± 1.7	9.8 ± 2.3	9.7 ± 1.9
IMPLANTATIONS	MEAN±S.D.	10.0 ± 1.7	9.4 ± 2.0	9.5 ± 1.8	9.7 ± 2.3	8.9 ± 2.4
LITTER SIZES	MEAN±S.D.	9.3 ± 1.8	8.8 ± 2.3	9.2 ± 1.7	9.5 ± 2.2	8.4 ± 1.9
LIVE FETUSES	N	168	176	156	57	101
	MEAN±S.D.	9.3 ± 1.8	8.8 ± 2.3	9.2 ± 1.7	9.5 ± 2.2	8.4 ± 1.9
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.7 ± 0.8	0.6 ± 0.9	0.4 ± 0.5	0.2 ± 0.4	0.5 ± 0.8
EARLY RESORPTIONS	N	9	9	5	1	1
	MEAN±S.D.	0.5 ± 0.8	0.4 ± 0.8	0.3 ± 0.5	0.2 ± 0.4	0.1 ± 0.3
LATE RESORPTIONS	N	3	2	1	0	5
	MEAN±S.D.	0.2 ± 0.4	0.1 ± 0.3	0.0 ± 0.2	0.0 ± 0.0	0.4 ± 0.8
DOES WITH ANY RESORPTIONS	N(%)	9(50.0)	7(35.0)	6(35.3)	1(16.7)	4(33.3)
DOES WITH ALL CONCEPTUSES RESORBED	N	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	18(100.0)	20(100.0)	17(100.0)	6(100.0)	12(100.0)
PLACENTAE APPEARED NORMAL	N(%)	18(100.0)	20(100.0)	17(100.0)	6(100.0)	12(100.0)

a. Dosage occurred on days 7 through 19 of gestation.

Offspring (malformations, variations, etc.):

Fetal evaluation was based on the live fetuses in the litters (as listed in the tables below, excerpted from part of Table 11, page 46 and Table 12, page 48 of the report and page 38 of the report Appendix G).

Body weight: There were no test article-related effects on fetal body weight.

Sex Ratio: There were no test article-related effects on fetal sex ratios.

External and Visceral malformations and variations:

There were no test article-related gross external or soft tissue alterations. Most findings from external and soft tissue examinations of fetus were either comparable to the control, within the historical control range (sponsor provided, Appendix H in the report), or was a single incidence at low doses. As shown in the table below for Study (b) .2204-002 (excerpted from part of Table 11, page 46 and Table 12, page 48 of the report), head snout short observed in 2 fetuses (2%) from the 2 mg/kg group and low-set kidney observed in a single 5 mg/kg fetus (0.6%) were not considered test article-related alterations due to lack of dose-dependency and low incidence.

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Study (b)2204-002

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a	I 0 (VEHICLE)	II 1	III 5	IV 10	V 2
LITTERS EVALUATED	N 18	20	17	6	12
FETUSES EVALUATED	N 168	176	156	57	101
LIVE	N 168	176	156	57	101
HEAD: SNOUT SHORT					
LITTER INCIDENCE	N(%) 0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(9.3)
FETAL INCIDENCE	N(%) 0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(2.0)**d,e
KIDNEYS: LOW-SET					
LITTER INCIDENCE	N(%) 0(0.0)	0(0.0)	1(5.9)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%) 0(0.0)	0(0.0)	1(0.6)	0(0.0)	0(0.0)

FOOTNOTES:

- a. Dosage occurred on days 7 through 19 of gestation.
- b. Fetus S423-6 had other gross external observations.
- c. Fetus S431-3 had other gross external observations.
- d. Fetus S476-8 had other gross external observations.
- e. Fetus S476-11 had other gross external observations.
- ** Significantly different from the vehicle control group value (p<0.01).

Study BXA00008

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a	I 0 (VEHICLE)	II 2
LITTERS EVALUATED	N 20	19
FETUSES EVALUATED	N 185	175
LIVE	N 185	175
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%) 14(70.0)	12(63.2)
FETUSES WITH ANY ALTERATION OBSERVED	N(%) 29(15.7)	25(14.3)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S. D. 15.1 ± 14.7	14.1 ± 15.6

- a. Dosage occurred on days 7 through 19 of gestation.

Skeletal malformations and variations: There were no test article-related skeletal alterations. The skeletal findings were either comparable to the control, within the historical control range (sponsor provided, Appendix H in the report), or lacked dose-dependency with one or two fetal incidences in the lower dose groups.

Study title: Subcutaneous Dosage-Range Development Toxicity Study of DX-88 in Rabbits

Key study findings:

Two female rabbits in the 20 mg/kg/day dosage group were sacrificed due to the severity of ulcerations at the injection site. Ulceration on the left or right flank, and discoloration, ulceration, and/or erythema, edema or scab at the injection sites were observed in these two rabbits. Test article-related clinical observations were limited to the injection site reactions which included scabs, ulceration, swelling, erythema or edema at the injection site(s) with various incidences in the test article-treated groups. The NOAEL for maternal systemic toxicity was identified at 10 mg/kg.

Under the conditions of this study, there were no test article-related embryo-fetal toxicity observed at the dose levels up to 20 mg/kg (AUC_{inf} = 9492 µg.min/mL).

Note: As a dose-range finding study, the number of pregnant rabbits per group was limited and the fetal visceral and skeletal examinations were not conducted. This study has limited utility in assessing the effects of DX-88 on embryofetal development in rabbits.

Study no.: (b) (4) 00031

Volume #, and page #: Electronic submission

Conducting laboratory and location:

(b) (4)

Date of study initiation: May 16, 2006

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: DX-88 in PBS, liquid, Lot# 227-03-001, purity: 94.93% (99.51% for product).

Methods

Doses: Subcutaneous doses of 0, 2, 5, 10, and 20 mg/kg from day 7 through 19 of gestation.

Species/strain: Timed-mated female rabbits/Hra:(NZW)SPF (b) (4)
(b) (4) The day of mating was considered to be GD 0

Number/sex/group: Six timed-mated female rabbits/group for main study.

Route, formulation, volume, and infusion rate: Subcutaneous administration, vehicle was PBS. Dose volume was 2.5, 0.2, 0.5, 1.0 and 2.0 ml/kg for dose levels of 0 (vehicle), 2, 5, 10 and 20 mg/kg, respectively. Injection sites were rotated among six equal areas.

Satellite groups used for toxicokinetics: Additional six timed-mated rabbits/group were assigned for dose groups of 2, 5, 10, and 20 mg/kg and were dosed once on GD 7.

Study design: Presumed-pregnant rabbits were administered subcutaneously once daily at doses of 0, 2, 5, 10 and 20 from GD 7 through 19. The surviving rabbits assigned for TK assessment were sacrificed after the last blood sample collection on GD 8. All main surviving rabbits were sacrificed on GD 29.

Parameters and endpoints evaluated: The dams were evaluated for survival (twice/day), clinical observations, abortion and premature delivery and deaths, body weight and food consumption (daily during the dosing and postdosing period). Blood samples were collected from main animals at approximately 0.5, 1, 2, 4, 8 and 16 hours post dose on GD 7 for APTT evaluation, and prior to and at approximately 2 hours post dose on GD 19 for drug concentration analysis. Blood samples were collected from TK animals at approximately 0.25, 0.5, 1, 2, 4, 8, 16 and 24 hours post the 1st dose. Plasma concentrations of DX-88 were measured by

(b) (4) using a validated ELISA method (Validation Report No. (b) (4)0225). All surviving main rabbits were sacrificed on GD 29 and Cesarean-sectioned and gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of corpora lutea, implantation sites, and uterine contents were examined. Fetuses were individually weighed, and evaluated for gender and gross external alterations. There were no examinations for visceral or skeletal malformations or variations.

Results

Mortality (dams): Two female rabbits in the 20 mg/kg/day dosage group were sacrificed due to the severity of ulcerations at the injection site. Adverse clinical signs of these two rabbits included ulceration on the left or right flank, and discoloration, ulceration, and/or

erythema, edema or scab at the injection sites. No remarkable body weight gains and feed consumption values changes throughout the study. Necropsy confirmed the ulceration at the injection sites and the flank in both rabbits and revealed a subcutaneous gelatinous dark red material at the umbilical area and at injection sites, and a white area at the septum of the ventral surface of the heart in one rabbits. No other significant necropsy findings were reported. The litter consisted of seven or nine embryos. All other rabbits survived to the scheduled termination.

Clinical signs (dams): All rabbits in the test article-treated groups had purple, red, yellow and /or black discoloration at the injection site(s). Four rabbits in the vehicle control group had injection site discoloration also. Additional signs of injection site irritation included scabs, ulceration, swelling, erythema or edema at the injection site(s) of the test article treated rabbits with various incidence rates. No other test article-related clinical signs were observed.

Body weight (dams): During the dosing period (calculated as GDs 7 to 20), body weight gains were 0.30, 0.34, 0.33, 0.25 and 0.20 kg in the 0, 2, 5, 10 and 20 mg/kg groups, respectively, indicating a slight reduction in the 10 and 20 mg/kg groups. Average body weights were comparable in all dose groups at the end of dosing period or at the termination.

Food consumption (dams): There were no significant food consumption changes observed. Absolute food consumption values (g/day) was slightly reduced by 10% in the 20 mg/kg group during the dosing period (calculated as GDs 7-20) when compared to the control, but was comparable to the control during GDs 20- 29 or GDs 7-29.

APTT Evaluation: Transient APTT prolongation was observed in the test article-treated groups at 30 minutes and 1 hour after dosage without a dose-dependent pattern. As shown in the table below (excerpted from page 14 of the report), the prolonged APTT lasted longer in the 20 mg/kg group, up to 8 hours post dose. All values were comparable at 16 hours post dose.

Dosage (mg/kg/day)	0 (Vehicle)	2	5	10	20
N	3	3	3	3	3
30 minute	42.25 ± 6.29 [2]a	58.70 ± 17.83	88.53 ± 9.34	81.23 ± 3.45	92.40 ± 0.00 [1]a
1 hour	61.03 ± 3.23	79.50 ± 2.03	89.63 ± 33.41	100.93 ± 12.72	79.00 ± 22.20 [2]a
2 hour	61.55 ± 11.67 [2]a	57.43 ± 13.47	83.50 ± 7.37	61.00 ± 25.21	94.40 ± 0.00 [1]a
4 hour	50.17 ± 6.05	54.33 ± 15.04	62.17 ± 9.66	49.77 ± 4.47	78.95 ± 6.43 [2]a
8 hour	55.50 ± 9.70	65.40 ± 0.00 [1]a	41.40 ± 8.20 [2]a	59.53 ± 21.32	80.05 ± 0.35 [2]a
16 hour	49.75 ± 11.53 [2]a	49.33 ± 13.57	55.40 ± 14.53	55.43 ± 4.97	53.60 ± 0.00 [1]a

[] = Number of values averaged.

a. Excludes values for samples that were clotted.

Toxicokinetics: TK parameters were summarized in the table below (excerpted from page 15 of the study report). One rabbit in the 5 mg/kg TK group was not pregnant. All rabbits for TK evaluation were included in the TK analysis. Half-life increased in a dose-dependent manner. In agreement with increasing half-life, clearance decreased in a dose-dependent manner. Volume of distribution increased in a dose-dependent manner and

indicated significant distribution of DX-88 into tissues. Cmax and AUC values increased in an approximate dose dependent manner.

	Group II 2 mg/kg/day	Group III 5 mg/kg/day	Group IV 10 mg/kg/day	Group IV 20 mg/kg/day
N	6	6	6	6
T _{1/2} (min)	76.2 ± 34.8	121 ± 26.6	140 ± 43.0	234 ± 100
Cl (mL/min/kg)	3.96 ± 0.41	3.08 ± 0.90	2.76 ± 0.99	2.36 ± 0.73
Vd (mL/kg)	449 ± 245	513 ± 78.0	589 ± 382	795 ± 406
C _{max} (mcg/mL)	(b) (4)			
C _{max} /Dose (kg*mcg/mL/mg)				
T _{max} (min)				
AUC _{0-inf} (mcg*min/mL)				
AUC _{0-inf} /Dose (mcg*min*kg/mL/mg)	509 ± 52.4	1710 ± 369	4050 ± 1508	9492 ± 4150
	254 ± 26.2	342 ± 73.8	405 ± 151	475 ± 207

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

There were no test article-related gross lesions revealed in the scheduled maternal necropsy. The gross lesions for the 20 mg/kg rabbits early sacrificed due to injection site reactions were described in the section of "Mortality". One doe in the 10 mg/kg/day dosage group had thickened walls of the left uterine horn with a red area on the wall. This doe (7524) had a unilateral pregnancy with conceptuses present only in the right uterine horn.

All rabbits were pregnant. C-section observations were based on 6, 6, 6, 6 and 4 pregnant does with ≥ 1 live fetuses in the 0, 2, 5, 10 and 20 mg/kg dose groups, respectively. There were no significant test article-related effects on C-sectioning or litter parameters. No doe had a litter consisting of only resorbed conceptuses, and there were no dead fetuses.

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	2	5	10	20
RABBITS TESTED	N	6	6	6	6	6
PREGNANT	N (%)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)
SACRIFICED DUE TO ADVERSE CLINICAL OBSERVATIONS	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (33.3)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	6	6	6	6	4
CORPORA LUTEA	MEANS.D.	9.3 ± 1.9	10.3 ± 1.9	9.3 ± 2.3	8.3 ± 1.4	9.8 ± 1.0
IMPLANTATIONS	MEANS.D.	9.0 ± 2.4	9.8 ± 1.5	9.0 ± 2.1	8.3 ± 1.4	9.5 ± 0.6
LITTER SIZES	MEANS.D.	8.8 ± 2.3	9.7 ± 1.4	8.5 ± 1.9	8.2 ± 1.5	9.5 ± 0.6
LIVE FETUSES	N	53	50	51	49	38
	MEANS.D.	8.9 ± 2.3	9.7 ± 1.4	8.5 ± 1.9	8.2 ± 1.5	9.5 ± 0.6
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEANS.D.	0.2 ± 0.4	0.2 ± 0.4	0.5 ± 0.5	0.2 ± 0.4	0.0 ± 0.0
EARLY RESORPTIONS	N	1	1	0	1	0
	MEANS.D.	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
LATE RESORPTIONS	N	0	0	3	0	0
	MEANS.D.	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
DOES WITH ANY RESORPTIONS	N (%)	1 (16.7)	1 (16.7)	3 (50.0)	1 (16.7)	0 (0.0)
DOES WITH ALL CONCEPTUSES RESORBED	N (%)	0	0	0	0	0
DOES WITH VIABLE FETUSES	N (%)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	4 (100.0)
PLACENTAE APPEARED NORMAL	N (%)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	4 (100.0)

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Offspring (malformations, variations, etc.):

Body weight: Fetal body weights were comparable among five dose groups.

Sex Ratio: Percent of live male fetuses were comparable among five dose groups.

External malformations and variations:

There were no test article-related gross external alterations.

Note: No examinations for visceral and skeletal malformation and variations were conducted.

Prenatal and postnatal development**Study title: Developmental and Perinatal/Postnatal Reproduction Toxicity Study of DX-88 Administered Subcutaneously in Rats, Including A Postnatal Behavioral/Functional Evaluation****Key study findings:**

Test article-related irritation at injection sites was evident at all dose levels. One 25 mg/kg F0 rat was sacrificed early due to severe ulceration at the injection site. There were no effects of DX-88 on the F0-generation dams at any dose level tested. The NOAEL for dams was 25 mg/kg.

There were no test article-related effects on F1 generation rats with respect to viability, growth, and development. The NOAEL for the viability and growth for F1 offspring was identified at the 25 mg/kg maternal dose. F2 fetuses were unaffected.

Study no.: (b) (4), 00006

Volume #, and page #: Electronic submission

Conducting laboratory and location:

(b) (4)

Date of study initiation: January 12, 2000

GLP compliance: Yes.

QA reports: Yes

Drug, lot #, and % purity: DX-88 drug product in PBS, pH 7, Lot # 227-05-001, purity, 99.58% (product) 94.54% (DX-88)

Methods

Doses: 0, 5, 10, and 25 mg/kg administered daily

Species/strain: Rats (b) (4): CD®(SD)

(b) (4)

Age at delivery: approximately 62 days

Body weight at study assignment: 211~246 g (females)

Number/sex/group: 25 mated female rats/group

Route, formulation, volume, and infusion rate: F0 rats were dosed by the subcutaneous route as shown in the table below.

Dosage Group	Dosage* (mg/kg/day)	Nominal Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned Rat Numbers
I	0 (Vehicle)	0	2.5	25	6501 - 6525
II	5	10.0	0.5	25	6526 - 6550
III	10	10.0	1.0	25	6551 - 6575
IV	25	10.0	2.5	25	6576 - 6600

a. The test article was considered 100% active for the purpose of dosage calculations.

Satellite groups used for toxicokinetics: No.

Study design: After acclimation, female rats were cohabitated with male rats. The cohabitation period was a maximum of 5 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be at GD 0 and assigned to individual housing. Presumed-pregnant female rats were dosed once daily from GD 7 through Lactation Day (LD) 20 or GD 24 (for rats that did not deliver a litter) for a total of 18 to 35 doses. Dams in the process of delivering pups were not dosed until completion of parturition. All dams were allowed to deliver naturally. F1 pups were not directly dosed with the test article/vehicle.

Parameters and endpoints evaluated:

For F0 rats, observations for clinical signs, abortions, premature deliveries and deaths were made daily before and after dosage administration, and on the day sacrifice occurred. Body weights were recorded on GD 0, daily during the dosage period and at sacrifice. Food consumption values were recorded on GDs 0, 7, 10, 12, 15, 18, 20 and 25 and LDs 1, 4, 7, 10 and 14. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes and pup viability at birth. Maternal behavior was evaluated on LDs 1, 4, 7, 14 and 21. On LD 21, F₀-generation rats were sacrificed and a gross necropsy was performed. The number and distribution of implantation sites were recorded.

For F1 pups during the preweaning period, each litter was evaluated for viability at least twice daily. The pups in each litter were counted once daily. Clinical observations were recorded once daily. Pup body weights were recorded on LDs 1, 4, 7, 14 and 21. On LD 21, randomly selected F₁-generation pups from each litter were continued on the study (25/sex/group) and the rest of the F1 pups were sacrificed and examined for gross lesions. At least one male pup and one female pup per litter, when possible, were selected.

For F1 generation rats during the postweaning period, clinical observations were recorded at least once weekly. Body weights for male rats were recorded weekly and at termination. Body weights for female rats were recorded weekly during the postweaning period and on GDs 0, 7, 10, 14, 17 and 21. Food consumption values for male rats were recorded twice weekly during the postweaning period, except during cohabitation. Food consumption values for female rats were recorded twice weekly during the postweaning period except during cohabitation and on GDs 0, 7, 10, 14, 17 and 21. Male and female rats were evaluated for sexual maturation and for learning and memory in passive avoidance (one male and one female from each litter, beginning at 24 ± 1 day postpartum) and water maze tests

(one male and one female from each litter, beginning at approximately 70 days postpartum). At approximately 90 days of age, the F1 generation rats were assigned to a 21-day cohabitation period. All surviving male rats were sacrificed after completion of the cohabitation period and a gross necropsy was performed. Testes and epididymides weights were recorded. All surviving female rats were sacrificed on GD 21, Caesarean-sectioned and a gross necropsy was performed. Each F2 fetus was weighed and examined for sex and gross external alterations.

Results

F₀ in-life: One rat (No. 6581) in the 25 mg/kg group was sacrificed due to the severity of the ulceration at the injection site on LD 5. Adverse clinical observation for this rat included discoloration, scabbing, and ulceration at the injection site. Pale extremities and an umbilical hernia were first observed at the necropsy on LD 5. The necropsy revealed a gelatinous material located subcutaneously at the injection site D. No other lesions were reported. Body weight gains appeared comparable to the control through gestation period and until LD 4.

At all dose levels, there were test article-related clinical signs consisting of irritation at the injection sites (discoloration, scabs or ulceration) (listed in the table below, excerpted from pages 47-48 of the report). Severity/incidence was increased and onset was accelerated with increased dosage and increased numbers of injections.

Injection Site	0 Injections	1 Injection	2 Injections	3 Injections
INCIDENTAL OBSERVATIONS				
PRENATAL OBSERVATIONS				
MATERNAL SURVIVAL INCIDENCE	37/37	35/37	27/37	30/37
INJECTION SITE(S): DISCOLORATION	0/0	0/0	2/2	12/12**
INJECTION SITE(S): SCAB(S)	0/0	1/2	3/3	7/7**
POSTNATAL				
MATERNAL SURVIVAL INCIDENCE	35/37	13/23	30/37	15/23
INJECTION SITE(S): DISCOLORATION	0/0	1/2**	11/11**	14/14**
INJECTION SITE(S): SCAB(S)	0/0	1/2	4/4	7/7**
INJECTION SITE(S): ULCERATION	0/0	1/2	4/4	11/11**

There were no other test article-related maternal deaths or clinical signs. There were no test article-related changes in maternal body weights, body weight gains or food consumption. As shown in the tables below (excerpted from pages 58-59 of the report), there were no test article-related effects on any natural delivery and litter observations.

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TABLE A11 (PAGE 11): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION RATS

DOSE GROUP		I		II		III		IV	
DOSE (MG/KG/DAYS)		0 (VEHICLE)		5		10		25	
RATS ASSIGNED TO NATURAL DELIVERY		25		25		25		25	
PREGNANT		24 (96.0)		23 (92.0)		24 (96.0)		23 (92.0)	
DELIVERED LITTERS		24 (100.0)		23 (100.0)		24 (100.0)		23 (100.0)	
DURATION OF GESTATION ^a MEANS ± S.D.		22.5 ± 0.5		22.6 ± 0.5		22.7 ± 0.5		22.7 ± 0.4	
IMPLANTATION SITE ^b PER DELIVERED LITTER MEANS ± S.D.		330		330		334		342	
DAYS WITH STILLBORN PUPS		0 (0.0)		0 (0.0)		3 (12.5)		4 (17.4)	
DAYS WITH NO LIVERBORN PUPS		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
GESTATION INDEX ^c		100.0		100.0		100.0		100.0	
DAYS WITH ALL PUPS DYING		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
DAYS 1-4 POSTPARTUM		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
DAYS 5-21 POSTPARTUM		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	

a. Days occurring on day 7 of gestation through day 20 of lactation.
 b. Calculated on days on the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the time the first pup was delivered.
 c. Number of rats with live offspring/number of pregnant rats.

The number of pups found dead or cannibalized during LD 8-14 in the 5 mg/kg dose group was increased compared to the control but decreased (0%) in the next period. The finding was isolated and not in a dose-dependent pattern, hence, was not considered toxicologically significant. There were no clinical signs, body weight changes and necropsy observations in pups attributed to maternal test article treatment. The slight dilation of the renal pelvis in one pup in the 25 mg/kg group was observed at necropsy on LD 21, which was not considered toxicologically significant due to the single incidence.

TABLE A12 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSE GROUP		I		II		III		IV	
DOSE (MG/KG/DAYS)		0 (VEHICLE)		5		10		25	
DELIVERED LITTERS WITH ONE OR MORE LIVERBORN PUPS		24		23		24		23	
PUPS DELIVERED (TOTAL)		346		323		330		318	
LIVERBORN		34.6 ± 1.0		34.0 ± 2.0		34.7 ± 2.2		33.0 ± 1.9	
STILLBORN		0.1 ± 0.3		0.0 ± 0.0		0.1 ± 0.3		0.2 ± 0.4	
LIVERBORN VITAL STATUS		0		1		0		0	
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED		0		1		0		0	
DAY 1		0/343 (0.0)		0/322 (0.0)		0/336 (0.0)		0/318 (0.0)	
DAYS 2-4		1/143 (0.7)		0/323 (0.0)		0/336 (0.0)		0/318 (0.0)	
DAYS 5-7		2/343 (0.6)		0/323 (0.0)		0/336 (0.0)		0/318 (0.0)	
DAYS 8-14		1/343 (0.3)		0/323 (0.0)		0/336 (0.0)		0/318 (0.0)	
DAYS 15-21		0/343 (0.0)		0/323 (0.0)		0/336 (0.0)		0/318 (0.0)	
VIABILITY INDEX ^a		99.7		99.7		100.0		99.0	
LACTATION INDEX ^b		342/343		321/323		335/336		310/318	

a. Days 1-7 of gestation.
 b. Days occurring on day 7 of gestation through day 20 of lactation.
 c. Includes values for litter sizes, day was sacrificed on day 8 of lactation due to the severity of injection site reactions.
 d. Number of live pups on day 1 postpartum/number of liverborn pups on day 1 postpartum.
 e. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 1 postpartum.
 f. Significantly different from the vehicle control group value (p < 0.05).
 g. Significantly different from the vehicle control group value (p < 0.01).

F0 necropsy: As described above, a gelatinous material located subcutaneously at an injection site of the rat early sacrificed due to the ulceration. There were no test article-related necropsy observations for the survival F0 rats.

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F₁ physical development: There were no test article-related mortalities in the F₁ rats. One male and one female F₁ rats in the 10 mg/kg groups were sacrificed on day 100 and day 73 postweaning, respectively. The clinical sign of red perioral substance, body weight loss (-65 g for the male during days 92-99, -30 g for the female during days 67-71) and reduced food consumption (female only during days 67-71) were found in these two rats and the early sacrifice was attributed to injuries to the incisors (misaligned). The other clinical signs (including, but not limited to, missing or misaligned incisors, chromodacryorrhea and/or red substance in the cage) observed in these two rats were also found in the control groups. One female F₁ rat in the 25 mg/kg group began to deliver a litter and was sacrificed on day 21 of gestation. All other F₁-generation rats survived to the scheduled termination.

There were no clinical signs or necropsy observations in the surviving F₁ male and female rats attributed to the test article. A yellow adipose mass of the abdomen in one 10 mg/kg male, a red adipose mass in one 25 mg/kg male and faccid left testis in one 25 mg/kg male were not considered toxicologically significant findings due to the single incidence and the nature of the observations.

The increases in male rat terminal body weights were 3%, 6% and 9% and the increase in body weight gains from day 1 through termination were 27% (+11g), 72% (+29 g) and 110% (+45 g) at doses of 5, 10 and 25 mg/kg, respectively, when compared to the controls. Similar trend was observed in the period of postweaning days 1-71 and of postweaning day 1 to precohobitation. However, the increase in body weight without any associated findings was not considered a test article-related adverse effect. No significant maternal test article related effects on body weights and body weight gains were observed in F₁ females. There were no test article-related changes in food consumption. No significant differences in absolute and relative weights of the testes and epididymides were observed in the F₁-generation male rats between maternal control and treated groups.

F₁ behavioral evaluation: There were no maternal test article treatment-related changes for learning, short-term retention, long-term retention and response inhibition in the F₁-generation rats, as evaluated by the performance in a passive avoidance paradigm and the water maze performance. In Session 2 test of errors per trial for trials to criterion, a statistically significant reduction was observed in the 10 mg/kg males and a statistically significant increase was observed in the 10 mg/kg females. Since the observation was not dose-dependent and not consistent between genders and test sessions, it was unlikely to be a test article-related adverse effect.

F₁ reproduction: There were no maternal test article treatment-related changes in the age of preputial separation in F₁-generation male rats or vaginal patency in female rats. The mating and fertility parameters were shown in the tables below (excerpted from page 169 of the report). The fertility index and the number of pregnancies per number of rats in cohobitation were reduced in the 5 mg/kg group compared to the control and other groups. As this reduction was not observed in the mid and high dose groups, it appeared unlikely to be a significant test article-related finding. Other mating and fertility parameters were comparable among control and treated groups.

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TABLE B21 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS.

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	10	25
RATS IN COHABITATION ^a	N	25	25	24 ^a	24 ^b
DAYS IN COHABITATION ^c	MEANS, D.	4.0 ± 3.5	3.2 ± 3.7	4.0 ± 2.6	3.0 ± 2.2
RATS THAT MATED ^d	N(N) (%)	23(92.0)	23(92.0)	23(95.8)	24(100.0)
FERTILITY INDEX ^{e, f}	N/N (%)	23/23 (100.0)	18/23 (78.3)**	23/23 (100.0)	23/24 (95.8)
RATS WITH CONFIRMED MATING DATES	N	23	22	23	24
MATED WITH FEMALE ^g					
DAYS 1-7	N(N)	22(95.6)	21(95.4)	22(95.6)	23(95.8)
DAYS 8-14	N(N)	1(4.3)	1(4.5)	1(4.3)	1(4.2)
RATS PREGNANT/RATS IN COHABITATION ^f	N/N (%)	23/25 (92.0)	18/25 (72.0)**	23/24 (95.8)	23/24 (95.8)

() = NUMBER OF VALUES AVERAGED
^a Excludes values for rat 7600, which was not assigned to cohabitation because there were no available female rats.
^b Excludes values for rat 7601, which was not assigned to cohabitation because there were no available female rats.
^c Restricted to rats with a confirmed mating date and rats that did not mate.
^d Includes only one mating for each male rat.
^e Number of pregnancies/number of rats that mated.
^f Includes only one pregnancy for each rat that impregnated more than one female rat.
^g Restricted to rats with a confirmed mating date.
^{**} Significantly different from the vehicle control group value (p<0.01).

F₂ findings: There were no effects on caesarean-sectioning parameters in the F₁-generation dams or the F₂-generation litters. The statistically significant increase in percent live male fetuses/litter in the 5 mg/kg maternal dose group was not considered a test article-related adverse effect. There were no fetal gross external alterations in the F₂-generation fetuses.

2.6.6.7 Local tolerance

Study (b)-446008: A Local Tolerance Study of Subcutaneously Administered DX-88 (liquid) in Rats

Key study findings:

The test article-related local gross and histopathology findings, primarily in females, were limited to the injection sites and resolved by day 14. The subcutaneous administration of 24.0 mg/kg DX-88 is tolerated by rats.

Study no.: (b)-446008

Volume #, and page #: EDR, section 4.2.3.6

Conducting laboratory and location:

Date of study initiation: November 8, 2004

GLP compliance: GLP compliance statement included

QA reports: yes (X) no ()

Drug, lot #, and % purity: DX-88, Lot# 227-01-004, purity 99.6% (DX-88 94.6%)

Formulation/vehicle: DX-88 in PBS pH 7.0/vehicle (PBS)

Methods

As shown in the table below, n=36/sex/group SD rats were subcutaneously administered once with 0 (vehicle, PBS), or 24 mg/kg DX-88 followed by an observation period up to 14 days. 5 rats/sex/group were euthanized on study days 1, 3, 5 and 10, and 8 rats/sex/group were euthanized on study days 7 and 14. The animals were observed twice

daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed twice prior to the initiation of dosing and on the days of scheduled necropsy. Individual body weights were recorded twice prior to the initiation of dosing, on the day of dosing and on the days of scheduled necropsy; individual food consumption was recorded weekly. Complete necropsies were conducted on all animals at the scheduled necropsies. Selected tissues (injection sites, gross lesions, liver, kidneys and spleen) were examined microscopically from all animals.

Group Number	Test Article	Dose Level ^a (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle	0	0	2.4	36	36
2	DX-88	24	10	2.4	36	36

^a = Dosage levels were based on the test article as supplied. No correction for purity was made.

Results:

All animals survived to the scheduled necropsies. There were no test article-related effects on body weights or food consumption. The test article-related clinical observations, gross and microscopic findings were limited to findings at the local injection sites. The clinical observations included scabbing, swelling and/or redness at the injection site, which occurred primarily in the female test article-treated group and began 2 to 3 days after dosing. Up to 60% females showed scabbing at injection sites from day 3 to 14. The gross pathology findings observed on the ventral surface of the excised skin surrounding the ecallantide injection sites, were dark red discoloration which were found in 8/36 males and 7/36 females treated with the test article; and scabbing which occurred in 1/36 males and 6/36 females treated with DX-88. These observations were generally noted within the first 3 days after test article administration. The microscopic findings as shown in the table below (excerpted from page 22 of the report) included minimal to mild hemorrhage and congestions at the injection sites and subacute inflammation confined to the dermis, underlying muscularis, and/or deeper subcutaneous adipose tissue. Less frequent findings were thrombosis, muscular degeneration and chronic inflammation. With the exception of exudate on the epidermal surface of one test article-treated group male at the study day 7 necropsy, all other epidermal lesions including exudate on the epidermal surface, squamous hyperplasia and ulceration were noted in females at 3 to 10 days after test article administration and were considered test article-related.

Text Table 1. Incidence Of Lesions At Injection Sites During Days 1-14 Post-Dosing

SEX	MALES												FEMALES											
	1		3		5		7		10		14		1		3		5		7		10		14	
GROUP	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
No/group	5	5	5	5	5	5	8	8	5	5	8	8	5	5	5	5	5	5	8	8	5	5	8	8
Hemorrhage	-	2	-	2	-	-	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-	-	-	-
Congestion	-	2	-	1	-	1	-	-	-	-	-	1	-	5	-	3	-	3	-	-	-	-	-	-
Inflammation, Subacute	-	3	-	3	-	3	-	-	-	1	-	-	-	4	-	3	-	2	-	1	-	4	-	-
Thrombosis	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inflammation, Chronic	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Exudate, Epidermal ¹	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	2	-	1	-	1	-	-	
Hyperplasia, Squamous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	1	-	-	
Degeneration, Muscle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	1	-	1	-	-	-	
Ulceration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-

¹Surface

Conclusion: The test article-related local gross and histopathology findings, primarily in females, were limited to the injection sites and resolved by day 14. The subcutaneous administration of 24.0 mg/kg DX-88 is tolerated by rats.

Study title: A Local Tolerance Assessment of Lyophilized DX-88 Subcutaneously Administered in Rats

Key study findings:

Test article-related clinical observation, gross and histopathology findings were limited to injection site alterations. The lesion included blood vessel necrosis, edema, inflammation, congestion, hemorrhage and epidermal necrosis and/or ulceration, which were more severe in females than males.

The injection sites injuries were completely resolved by day 14 in males while minor alterations were present in some females by day 14.

There was no evidence of systemic toxicity based on the organs evaluated microscopically.

Study no.: (b) 4460013

Volume #, and page #: EDR, section 4.2.3.6

Conducting laboratory and location:

Date of study initiation: November 14, 2005

GLP compliance: GLP compliance statement included

QA reports: yes (X) no ()

Drug, lot #, and % purity: DX-88, Lot# 05RP029, purity 99.6% (DX-88 94.9%)

Formulation/vehicle: DX-88 in 10 mM histidine, 10% sucrose

Methods

As shown in the table below (excerpted from page 15), n=30/sex/group SD rats were subcutaneously administered once with 0 (vehicle control), or 24 mg/kg DX-88 followed by a non-dosing period up to 14 days. Five rats/sex/group were euthanized on study days 1, 3, 5, 7, 10 and 14. The animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed on the days of scheduled necropsy. Individual body weights and food consumption were recorded on study day 0 and on the days of the scheduled necropsies. Complete necropsies were conducted on all animals at the scheduled necropsies. Selected tissues (injection sites, non-injection site skin, gross lesions, liver, kidneys and spleen) were examined microscopically from all animals.

Group Number	Test Article ^a	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Number of Animals	
				Males	Females
1	Placebo	0	0.8	30	30
2	DX-88 (lyophilized)	24	0.8	30	30

^a = Dosage levels were based on the test article as supplied. No correction for purity was made.

Results:

All animals survived to the scheduled necropsies. There were no test article-related

effects on body weights or food consumption. The test article-related clinical observations, gross and microscopic findings were limited to findings at the local injection sites. Test article-related clinical observations consisted of scabbing, swelling and/or redness at the injection site, which occurred primarily in the lyophilized DX-88-treated group females. These findings were noted as early as 3 days following dose administration and were observed through study days 3 (redness at the injection site), 5 (swelling at the injection site) or 10 (scabbing at the injection site). No animals exhibited overt signs indicative of pain at the injection sites upon palpation. Macroscopic observations included dark red areas and scabbing in the DX-88-treated group males and females. The microscopic findings as shown in the tables below (excerpted from page 23), revealed significant test article-related local injury. Females were more severely affected than males. All findings were resolved by study day 14 in males while minor alterations including hyperplasia, chronic inflammation, fibrosis, or regeneration were present in 1 to 4 females on study day 14. There was no evidence of systemic toxicity based on the organs evaluated microscopically.

Text Table 1: Male Incidence of Lesions at Injection Sites (5 animals/group/day)

Study Day Group	1		3		5		7		10		14	
	1	2	1	2	1	2	1	2	1	2	1	2
Inflammation, Subacute	1	5	-	1	-	-	-	2	-	-	-	-
Hyperplasia	-	3	-	3	-	3	-	1	-	1	-	-
Congestion	-	5	-	4	-	-	-	-	-	-	-	-
Hemorrhage	-	5	-	3	-	-	-	-	-	-	-	-
Necrosis, Vascular	-	5	-	2	-	-	-	-	-	-	-	-
Edema	-	4	-	2	-	-	-	-	-	-	-	-
Mineralization	-	1	-	1	-	1	-	2	-	1	-	-
Inflammation, chronic	-	-	1	4	1	5	-	-	-	2	-	-
Fibrosis	-	-	-	2	-	2	-	1	-	-	-	-
Exudate, epidermal surface	-	-	-	1	-	-	-	-	-	-	-	-
Inflammation, granulomatous	-	-	-	-	-	1	-	2	-	1	-	-
Regeneration	-	-	-	-	-	2	-	1	1	-	-	-

Text Table 2: Female Incidence of Lesions at Injection Sites (5 animals/group/day)

Study Day	1		3		5		7		10		14	
Group	1	2	1	2	1	2	1	2	1	2	1	2
Inflammation, Subacute	2	5	-	3	-	-	-	-	-	1	-	-
Hyperplasia	-	5	1	4	-	4	-	2	-	3	-	1
Congestion	-	5	-	4	-	-	-	-	-	-	-	-
Hemorrhage	-	5	-	3	-	-	-	-	-	-	-	-
Necrosis, Vascular	-	5	-	2	-	1	-	-	-	-	-	-
Edema	-	5	-	3	-	2	-	-	-	-	-	-
Ulceration	-	-	-	1	-	-	-	-	-	1	-	-
Mineralization	-	-	-	-	-	1	-	2	-	-	-	-
Inflammation, chronic	-	-	2	2	-	5	-	3	-	3	-	4
Fibrosis	-	-	-	-	-	4	-	2	-	4	-	2
Exudate, epidermal surface	-	3	-	3	-	2	-	1	-	3	-	-
Necrosis	-	1	-	2	-	1	-	-	-	-	-	-
Inflammation, granulomatous	-	-	-	-	-	-	-	2	-	-	-	-
Regeneration	-	-	-	-	-	2	-	2	-	2	-	1

Conclusion: The test article-related local gross and histopathology findings, primarily in females, were limited to the injection sites and resolved by day 14. The subcutaneous administration of 24.0 mg/kg DX-88 is tolerated by rats.

Study title: A Toxicokinetic and Injection Site Reaction Study of DX-88 Administered Subcutaneously as Liquid and Lyophilized Formulations in Minipigs (Study No. 446012)

The study report was previously reviewed in draft form by Dr. Jean Wu in IND 10426 SN098 and SN106 attached (see Appendix 1). The final report has been submitted in IND 10426 SN115 and in the current BLA. The sponsor indicated that there were no significant changes made between the draft report and the final report. In fact, the incomplete histopathology evaluation for the selected tissues was completed in the final report and did not reveal any significant test article-related systemic toxicity. The individual and summarized PK data were included in the final report and consistent with the preliminary PK summary. Therefore, the study conclusion was not altered.

2.6.6.8 Special toxicology studies

The following non-GLP studies were conducted to explore the mechanism of acute death observed in females after intravenously administration of DX-88 in some early stage toxicity studies in rats. The study results were summarized in the tables below (excerpted from EDR section 2.6.7.14).

Table 2.6.7.14. Other Toxicity Studies - Test Article: Ecallantide

Species / Strain	Method of Administration	Duration of Dosing	Dose	Gender and No. per Group	Noteworthy Findings	Study Number
In vitro Purkinje fibers from canine heart ventricles	In vitro perfusion	NA	0, 0.25, 2.5, 25 µM	Male	<p>Ecallantide to 25 µM did not prolong the action potential repolarization, and its contributing ion channels, in cardiac Purkinje fibers.</p> <p>APD₅₀: no prolongation at any basic cell length tested.</p> <p>APD₉₀: no prolongation of any basic cell length at 0.25 and 2.5 µM. At 25 µM no prolongation of 0.5 and 1 second, but prolongation of 2 second cycle vs. vehicle</p> <p>No changes in resting membrane potential, action potential amplitude or maximum rate of depolarization.</p>	(b) 050224(b) (4)
In vitro Single ventricular cardiomyocytes from Sprague-Dawley rat hearts	In vitro perfusion	NA	Male: 0, 30, 100 µM Female: 0, 10, 100 µM	Male and Female	Ecallantide to 100 µM did not inhibit the inward sodium current I _{Na} , or the transient outward potassium current I _{to} , in rat ventricular cardiac myocytes	051220 (b) (4)
Ex vivo Sprague-Dawley rat hearts	In vitro perfusion	NA	0, 0.25, 2.5, 25 µM	Female	Ecallantide to 25 µM had no effect on ECGs of left ventricular function in perfused, adult, female rat hearts.	GENZ 06GSTR077
Rat, Sprague Dawley	Intravenous, Subcutaneous (PBS)	1 day	0 and 30 mg/kg, SC 0, 20 30 and 50 mg/kg, IV	7 F (telemetered)	<p>Normal F rats with 30 mg/kg ecallantide SC had no qualitative changes in ECG.</p> <p>After ovariectomy, rats with IV 20 or 30 mg/kg ecallantide had no arrhythmias, but with 50 mg/kg, all had decreased blood pressure and 2 of 7 had ECG changes.</p> <p>After ovariectomy and supplemented with β-estradiol, rats with IV 30 mg/kg ecallantide had lowered blood pressure and no ECG changes. At 50 mg/kg 2 of 7 died with ECG conduction disturbances and 4 of 7 had qualitative cardiac changes.</p>	(h) (4) FFA00026

Species / Strain	Method of Administration	Duration of Dosing	Dose	Gender and No. per Group	Noteworthy Findings	Study Number
Rats, Sprague Dawley	Intravenous	1 day	30	5M (normal); 5F (normal); 5M (castrated); 5F (ovariectomized)	No effects in any males. Mortality: 2 of 5 normal F died 10 minutes post-dosing without prior clinical observations. No cause of death attributed. Four of 5 normal F were lethargic post-dosing. Two of 5 ovariectomized F were lethargic, 0 of 5 died. No cardiac lesions in acute deaths or survivors. Hepatocellular vacuolization common in all groups.	GENZ 05-0103PDX
Rat, Sprague Dawley	Intravenous (PBS)	4 days (3 escalating doses)	0, 5, 25 and 50 mg/kg	6 M and 6 F (telemetered)	Mortality: 2 of 6 F died of cardiac complications soon after IV dosing of 50 mg/kg. No necropsy. IV administration of 50 mg/kg in M, and 25 and 50 mg/kg in F, caused cardiovascular findings of sinus arrhythmia and arrest, atrial premature complexes, ventricular premature complexes, and second-degree AV block.	(b) (4) (b) (4) 0001
Rat/ Crl:CD(SD)	Intravenous (PBS)	1 day	0 5 mg/kg and 20 mg/kg (low -18D); 20 mg/kg (high -18D)	8M, 8F	Flushing of extremities at 20 mg/kg in M and F with low and high -18D ecallantide. ALT and AST liver enzymes elevated in 3 of 8 F at 20 mg/kg low -18D. No hepatic microscopic correlate. Toxicity profiles of low and high -18D considered comparable.	(b) -446009

2.6.7 TOXICOLOGY TABULATED SUMMARY

Toxicology tabulated summary of studies as provided by the sponsor are available at EDR.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS:

Ecallantide is a 60 amino acid recombinant protein inhibitor of plasma kallikrein developed as a potential treatment for hereditary angioedema (HAE). In vitro pharmacology studies demonstrated that ecallantide was a potent plasma kallikrein inhibitor with a Ki of 25 pM for the human form and a Ki of 17 nM for the rat form. Ecallantide inhibited plasma kallikrein in human and other species tested using activated endogenous plasma kallikrein. Nanomolar inhibition was demonstrated in human monkeys, pigs, rabbits, and dogs and micromolar inhibition in rats and mice. It is intended for the subcutaneous route of administration. In two *in vivo* animal models, ecallantide administered by the IV route reduced in a dose-dependent manner, increases of vascular permeability in C1-INH deficiency mice, and alleviated the carrageenan-induced paw swelling in rats at a subcutaneous dose of 30 mg/kg, which was similar to the effect of a bradykinin receptor antagonist, tested in the same model as a reference.

Following subcutaneous administration, the terminal half-life ranged from 143-158 minutes in rats, rabbits and minipigs. Following intravenous administration, the half-life ranged from 19-99 minutes in rats, monkeys, rabbits and minipigs. Due to the relatively short half-life, the repeat-dose PK profile was consistent with the single dose profile. Volume of distribution values in all species indicated ecallantide could distribute beyond the blood volume into the tissues. Generally, exposure increased with increasing dose in

both monkeys and rats with no consistent gender differences in disposition observed. The exposure appeared to be greater in the Cynomolgus monkey than in the rat.

The preclinical program included 6 month, repeat dose, subcutaneous toxicology studies in rats and monkeys as well as other short term toxicology studies. Reproductive toxicology assessment included a fertility study in rats, teratology studies in rats and rabbits, and a perinatal/postnatal study in rats. The carcinogenic potential of ecallantide has not been evaluated. A carcinogenicity study should be conducted in one species (rat) as a post-marketing commitment.

Effects of ecallantide observed in the nonclinical toxicology studies generally appeared to be extensions of the expected pharmacological effects of the product.

In the 6-month rat toxicity study, DX-88/vehicle was administered subcutaneously at dose levels of 0, 0.4, 10 and 25 mg/kg once every three days. Three unscheduled deaths occurred between SD 103 to 142 in the 25 mg/kg males and females and potential relationships to treatment could not be excluded; however, the incidence (4.7%) was low. For these 3 deaths, one female that died early showed multiple necrosis in the brain including optic chiasm, the lateral internal capsule and optic tract, the fimbria of the hippocampus and the ventral midline of the brainstem, and for the other 2 animals, the cause of the death could not be determined. One control group female also died during this period. There were significant increases of AST and ALT values observed in the 25 mg/kg females that resolved during the recovery period and were not associated with any liver lesions in microscopic examination. Test article-related injection sites reactions observed in both males and females of the 25 mg/kg group and in females only of the 10 mg/kg group included inflammation, epithelial hyperplasia, exudate, ulceration, and granulation tissue/fibrosis. These injection site reactions were completely resolved after recovery period in most animals except for the 25 mg/kg females that was only partially resolved. The NOAEL was identified at 10 mg/kg (approximately 5-fold of maximum recommended human dose on an AUC basis).

In monkeys, a 3-month study with daily sc dosing up to 25 mg/kg and a 6-month study with once every three day sc dosing up to 25 mg/kg were conducted. There was no significant evidence of systemic toxicity observed in either study. Test article-related effects were limited to the injection sites, which included minimal to moderate perivascular mixed inflammatory cell infiltrates at injection sites observed in males and females at doses of 10 and 25 mg/kg in both studies. The NOAELs for systemic toxicity in both monkey studies were identified at 25 mg/kg while the NOAELs for local toxicity would be 0.2 and 0.4 mg/kg for the 3- and 6-month studies, respectively.

In animal studies, ecallantide caused a dose-dependent, reversible prolongation of APTT, which is thought to be due to ecallantide inhibition of activation of factor XII to factor XIIIa in the clotting cascade. However, there was no evidence of gross bleeding in the animals with the increase in APTT.

With respect to immunogenicity, anti-ecallantide antibodies were noted in both rats and monkeys and at a higher frequency in the higher dose groups. Based upon the

pharmacokinetic data, clearance of ecallantide was reduced and systemic exposure was increased following the development of ecallantide antibodies. However, there was no increase in toxicity noted with the higher exposure. Drug activity appeared to be maintained throughout the duration of the 6-month rat and monkey studies based upon measurements of elevated APTT.

NOAELs of the 6-month toxicology studies with ecallantide in rats and monkeys provide adequate safety margins on an AUC basis for the maximum recommended human dose of ecallantide (2 single 30-mg doses within 24 hours, which yields a total dose of 60 mg per person in one day).

Safety margins for the maximum recommended human dose of ecallantide (2 single 30-mg doses within 24 hours, which yields a total dose of 60 mg per person in one day equivalent to 1.2 mg/day or 44 mg/m²; AUC = 362 µg·min/mL).

General toxicity	route	mg/kg/d	*AUC factor	mg/m ²	Dose Ratio mg/kg	AUC	Rounded Dose Ratio mg/m ²
6-mon rat			6	150	20.8	9.4	3
6-mon rat			6	60	8.3	4.7	1
6-mon monkey			12	300	20.8	46.2	7

* AUC_{0-24hr} on SD 177

A complete battery of reproductive toxicity studies was conducted with DX-88. In the reproductive toxicity studies, subcutaneous doses of DX-88 in rats up to 25 mg/kg/day did not show any adverse effects on male or female fertility and reproductive functions. In the embryonic and fetal development studies with rats and rabbit, subcutaneous doses of DX-88 in rats up to 20 mg/kg/day did not result in any developmental toxicity. An embryofetal development study was conducted in rats with intravenous doses of 0, 10, 15, and 20 mg/kg/day. At 20 mg/kg/day, there were increased the number of early resorptions and the percentage of resorbed conceptuses per litter with corresponding reduced number of live fetuses, reduced fetal body weights (-11%), increased incidence of fetal alterations, including marked dilation of the lateral ventricle of the brain, short tail, vertebrate/rib malformations. These findings occurred in the presence of significant maternal toxicity. At 15 mg/kg/day, there were increased the number of early resorptions and the percentage of resorbed conceptuses per litter without significant reduced number of live fetuses and reduced fetal body weights (-4%) in the 15 mg/kg group. These findings occurred in the presence of relatively mild maternal toxicity and should be reported in the labeling. The development NOAEL was identified at 10 mg/kg (AUC_{inf}=7.55 µg·hr/mL= 453 µg·min/mL after single intravenous dose of 10 mg/kg). Intravenous doses of DX-88 in rabbits at dose levels of 2, 5 and 10 mg/kg did not show any adverse development toxicity. However, the number of rabbits at 10 mg/kg dose group (n=10) was not considered sufficient for a full evaluation. Subcutaneous doses of DX-88 up to 25 mg/kg in F₀ rats did not affect the gestation, parturition, or lactation in the F₀-generation rats, growth, development, and reproductive performance of the F₁-generation rats, and the viability and growth in the offspring. DX-88 was not measured in the milk of rats.

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Conclusions: The applicant has a complete nonclinical pharmacology and toxicology program for ecallantide, which supports the safety of the proposed clinical dose.

Unresolved toxicology issues (if any):

The carcinogenic potential of ecallantide has not been evaluated. A carcinogenicity study should be conducted in one species (rat) as a post-marketing commitment.

Recommendations: From a nonclinical perspective, approval is recommended for the application.

Labeling Review

Based on the Draft Guidance for Industry "Labeling for Human Prescription Drug and Biological Products – Implementing the New Content and Format Requirements" (January 2006), additional heading (i.e. 13.2 Animal Toxicology) was created.

For nonclinical sections 8.1 and 13.2 of the proposed labeling, increased number of early resorption and percentage of resorbed conceptuses per litter were observed in rats that received intravenous dose of 15 mg/kg/day (approximately 15 times MRHD on a mg/kg basis). These findings occurred in the presence of relatively mild maternal toxicity and should be reported in the labeling and Pregnancy Category C was recommended based on these findings. (b) (4)

[REDACTED] (b) (4)

Therefore, the result was not conclusive and should not be included in the labeling.

For section 10 in the proposed labeling, at least information from monkey studies should be included as monkey would be considered most relevant species to human.

For sections 12.1 and 12.2 in the proposed labeling, the mechanism of action should be based on human and animal data or specific references. (b) (4)

[REDACTED]

[REDACTED] (b) (4)



(b) (4)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of KALBITOR in pregnant women. KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Because animal reproductive studies are not always predictive of human response, KALBITOR should only be used during pregnancy

(b) (4)

(b) (4)

In rats, KALIBITOR at an intravenous dose approximately 13 times the MRHD on a mg/kg basis caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. No development toxicity was observed in rats that received an intravenous dose approximately 8 times the MRHD on a mg/kg basis. There were no adverse effects of KALIBITOR on embryofetal development in rats that received subcutaneous doses up to 2.4 times the MRHD on an AUC basis, and in rabbits that received intravenous doses up to 6 times the MRHD on an AUC basis.

(b) (4)

(b) (4)

(b) (4)

10 OVERDOSAGE

There have been no reports of overdose with KALBITOR. HAE patients have received single doses up to 90 mg intravenously without evidence of dose-related toxicity.

No deaths occurred in monkeys that received intravenous or subcutaneous doses up to 25 mg/kg (approximately 22 times the MRHD on an AUC basis)

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Hereditary angioedema (HAE) is a rare genetic disorder caused by mutations to C1-esterase-inhibitor (C1-INH) located on Chromosome 11q and inherited as an autosomal dominant trait.

C1-INH is a major endogenous inhibitor of plasma kallikrein. HAE is characterized by low levels of C1-INH activity and low levels of C4. In HAE, normal regulation of plasma kallikrein activity and the classical complement cascade is therefore not present. During attacks, unregulated activity of plasma kallikrein produces, through excessive bradykinin generation, the characteristic symptoms of localized swelling, inflammation, and pain.

KALBITOR is a potent ($K_i = 25\text{pM}$), (b) (4) and reversible inhibitor of (b) (4) plasma kallikrein. The kallikrein-kinin system is a complex proteolytic cascade involved in the initiation of both inflammatory and coagulation pathways. (b) (4)

(b) (4) By directly inhibiting plasma kallikrein, ecallantide reduces (b) (4) and thereby treats symptoms of the disease during acute episodic attacks of HAE.

12.2 Pharmacodynamics

No exposure-response relationships for KALBITOR to components of the complement or kallikrein-kinin pathways have been established. (b) (4)

The effect of KALBITOR on activated partial thromboplastin time (aPTT) was measured because of potential effect on the intrinsic coagulation pathway. Prolongation of aPTT has been observed following IV dosing of KALBITOR at doses $\geq 20\text{ mg/m}^2$. At 80 mg IV in healthy subjects, aPTT values were prolonged approximately two-fold over baseline values and returned to normal by 4 hours post-dose. (b) (4)

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

There are no animal or human studies to assess the carcinogenic or mutagenic potential of KALBITOR (ecallantide).

(b) (4)

13.2 Animal Toxicology

Reproductive Toxicology Studies

KALIBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Treatment of rats with an intravenous dose of 15 mg/kg/day (approximately 13 times the MRHD on a mg/kg basis) caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. However, no development toxicity was observed in rats that received an intravenous dose of 10 mg/kg/day (approximately 8 times the MRHD on a mg/kg basis). KALIBITOR had no adverse effects on embryofetal development in rats at subcutaneous doses up to 20 mg/kg/day (approximately 2.4 times the MRHD on an AUC basis) and rabbits that received intravenous doses up to 5 mg/kg/day (approximately 6 times the MRHD on an AUC basis).

Signatures (optional):

Reviewer Signature *J. Wu* 2/13/09
Jean Q. Wu, MD, PhD
2-13-09

Supervisor Signature *Timothy N. Robison* Concurrence Yes No
Timothy Robison, PhD, DABT

Appendix/Attachments

Appendix 1: Pharmacology/Toxicology Review of IND 10426 Serial No. 090 dated 9/30/2005

Appendix 2: Pharmacology/Toxicology Review of IND 10426 Serial No. 098 dated 1/17/2006

Drug: Ecallantide

Intermittent dose once every 72 hours

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age	mg/dose	AUC	doses	mg/day	mg/kg	AUC	factor	mg/m ²
Adult	>12			60	1.2	362	37	44.4
				conv.	Dose Ratio		Rounded Dose Ratio	
route	mg/kg/d	AUC	factor	mg/m ²	mg/kg	AUC	mg/m ²	
Reproduction and Fertility:								
rat			6	150	20.8	---	3	
Teratogenicity:								
#rat			6	120	16.7	2.4	3	
rat			6	90	12.5	---	2	
#rat			6	60	8.3	1.3	1	
*rabbit			12	60	4.2	5.5	1	
Overdosage:								
rat			6	150	20.8	---	3	
rat			6	60	8.3	---	1	
rat			6	144	20.0	---	3	
#mini pig			35	525	12.5	10.7	12	
**monkey			12	276	19.2	21.5	6	

AUC unit: µg.min/mL;

AUC value in the study = AUC_{0-inf}

* AUC value in the study = AUC_{0-last} or AUC_{0-360 min}

** AUC value in the study = AUC_{0-360 min} on Day 14

APPENDIX/ATTACHMENTS

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: BB-IND# 10426

Review number: N/A

Sequence number/date/type of submission: 090/September 30, 2005/Non-Clinical Information, 113/March 15, 2006 (received July 13, 2006)/Non-Clinical Information

Information to sponsor: Yes () No (X)

Sponsor and/or agent:

Dyax Corp.
300 Technology Square
Cambridge, MA 02139

Manufacturer for drug substance:

Reviewer name: Jean Q. Wu

Division name: Division of Pulmonary and Allergy Products

HFD #: 570

Review completion date: 7/18/06

Drug:

Generic name: Recombinant Human Plasma Kallikrein Inhibitor

Code name: DX-88

Chemical name: N/A

Relevant INDs/NDAs/DMFs: BB-IND# (b) (4)

Drug class: Biologics

Intended clinical population: Hereditary Angioedema Patients

Clinical formulation: 10 mg of DX-88 in 1 mL PBS, pH 7.0

Route of administration: Subcutaneous

Proposed clinical protocol: N/A. Based on the sponsor's letter of September 30, 2005, the draft report of study (b) -446005 was submitted in Serial #077 to support the change in administration of DX-88 in clinical trial DX-88/5 EDEDMA2 from intravenous to subcutaneous. In submission serial #077, the amendment 5 of the clinical trial was submitted and proposed change the dose from 10 mg/m² iv. to flat 30 mg sc with three injections of 1 mL (10 mg/mL) each. The treatment plan of the clinical protocol (amendment 5) in the submission #077 stated that patients were allowed a maximum of 20 treatments with DX-88 within DX-88/5 study. In the event of an incomplete response to the initial dose of DX-88, defined as a relapse or partial response at 4 to 24 hours after the 1st dose, another dose may be given. Patients may also be treated every four days for repeat attacks if all Day 7 follow up procedures are complete.

Previous clinical experience: 7 clinical studies. Only one conducted in healthy volunteers with subcutaneous dosing, in which the injection site inflammation, pruritus and rash were reported. (S-086, attachment 3)

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: Study No (b)-44605
Study title: An Acute Toxicity and Toxicokinetic Study of DX-88 Following Subcutaneous or Intravenous Administration in Mini-pigs.

This study draft report was submitted in serial #077.

Studies not reviewed within this submission: None

TABLE OF CONTENTS

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW.....	1
2.6.1 INTRODUCTION AND DRUG HISTORY.....	1
2.6.2 PHARMACOLOGY.....	4
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	4
2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....	4
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	4
2.6.6 TOXICOLOGY.....	4
2.6.6.1 Overall toxicology summary.....	4
2.6.6.2 Single-dose toxicity.....	4
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	8

2.6.2 PHARMACOLOGY

N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

N/A

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

2.6.6.2 Single-dose toxicity

Study title: An Acute Toxicity and Toxicokinetic Study of DX-88 Following Subcutaneous or Intravenous Administration in Mini-Pigs (Study No. (b)446005)

Key study findings:

No test article-related adverse effects on clinical observation, body weight, ECG, clinical pathology, gross pathology, and organ weight.

Lung edema found in 1/3 males of each test article-treated group and 2/3 females at 25 mg/kg sc group

Mild vasculitis at the injection site observed in one female of 15 mg/kg iv. group

NOAEL can not be identified

Study no.: (b)-446005

Volume #, and page #: Vol. 1-3. Pages 1-703

Conducting laboratory and location:

Date of study initiation: 9/10/2004, Date of the 1st dosing: 9/15/04

GLP compliance: GLP

QA report: yes (x) no ()

Drug, lot #, and % purity: DX-88, Lot No. 227-01-003 and Lot No. 227-01-004, purity for both lots: 99.6%

Methods

Doses: a single subcutaneous (s.c.) dose at 0, 15 and 25 mg/kg and a single iv. slow bolus at 15 mg/kg.

Species/strain: Hanford mini-pigs

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume, and infusion rate:

s.c bolus at two injection sites or i.v. over 10 minutes

DX-88 solution (supplied as frozen liquid) at 10 mg/mL, vehicle: PBS

Dose volume: 2.5 mL/kg for dose at 0 and 25 mg/kg, 1.5 mL/kg for dose of 15 mg/kg

Age: approximately 3.5 months old

Weight: Male, 11.5-16.8 kg, Female, 10.4-14.6 kg at the initiation of dosing.

Observation and Times:

Mortality and moribundity were assessed twice daily, and clinical observations were performed daily. Detailed physical examinations and body weight were recorded weekly. Hematology, clinical chemistry and urinalysis were evaluated prior to the initiation of dosing (Day -8), 2 and 24 hours post dose, and prior to the scheduled necropsy (Day 14). Activated partial thromboplastin time (APTT) was evaluated at 0 (pre-dose), and 0.25, 0.5, 1, 4, 8, 36, 48 and 72 hours post dose. ECGs were recorded during week -2, and week 2 (prior to the scheduled necropsy). Complete necropsy was performed on all animals sacrificed following 14 days observation. As listed in the inventory table at the end of section 2.6., selected organs were weighed and selected tissues from all animals were examined microscopically (without peer review).

Blood samples for TK evaluation were collected at 0 (pre-dose), 0.25, 0.5, 1, 4, 8, 36, 48 and 72 hours post dose.

Results:

Mortality: None.

Clinical signs: There were no test article-related clinical observations reported.

Body weights: There were no test article-related effects on body weights.

Hematology: APTT (activated partial thromboplastin time) was prolonged in all male and female treated groups. The data was listed in the table below. APTT prolongation occurred as early as 15 min post-dose in all treated groups. Mean APTT returned to comparable control values by 8 hours post-dose in 15 mg/kg iv. group, by 36 hours post-dose in 15 mg/kg sc. groups and by 24 hours post-dose in 25 mg/kg sc. groups. The transient prolongation in APTT was consistent with the pharmacologic effect of DX-88 involving kallikrein inhibition (Williams and Baird, 2003, Ritchie, 2003), hence, was not considered as an adverse effect.

Clinical Chemistry: No test article-related alterations were observed.

Urinalysis: No test article-related alterations were observed

EKG: There were no test article-related findings in qualitative ECG evaluation. No test article-related effect on heart rate was reported.

Gross pathology: No test article-related gross pathology findings were reported.

Organ weights: No test article-related organ weight changes were reported.

Histopathology: Mild to moderate lung edema was found in 1/3 males in each test article-treated group and 2/3 females at 25 mg/kg sc. group. There was no evident inflammation response related to the lung findings. As the concurrent control groups did not have similar findings, it is hard to determine if it is not the test article-related adverse effect. The mild to moderate granulomatous inflammation in sciatic nerve was found in both control and test article-treated groups. No lesion within nerve itself was reported. Due to the lack of dose response and the incidence in the control group, the inflammation was not considered as an adverse effect. One female in 15 mg/kg iv. group had a mild vasculitis at the injection site although the iv. dose occurred 14 days prior to the necropsy. Since the immediate reaction to the test article was not assessed, it is hard to exclude this finding as the test article-related effect. There were no test article-related findings at injection sites for all s.c. groups. Other microscopic findings were not considered as test article-related adverse effects.

Adequate Battery: yes (x), no ()—explain
Peer review: yes (), no (x)

Toxicokinetics: The TK parameters were shown in the table listed below.

Dose (mg/kg)		AUC _{0-∞} (µg.hr/mL)	T _{1/2} (hours)	C _{max} (ng/mL)	T _{max} (hours)	Cl (L/h/kg)
15 i.v.	Male	72530.5	2.2	36014.7	0.25	0.209
	Female	56007.3	1.1	31421.8	0.25	0.269
15 s.c.	Male	95220.0	3.3	9595.3	2.2	0.158
	Female	82651.6	3.2	11621.2	2.0	0.182
25 s.c.	Male	151088.7	2.6	13049.1	3.3	0.166
	Female	128977.7	2.4	12275.7	2.0	0.195

Histopathology inventory (optional)

Study	Single dose
Species	Mini-pig
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	X*
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	X

Eye	X	
Fallopian tube		
Gall bladder	X	
Gross lesions	X	
Harderian gland		
Heart	X*	
Ileum	X	
Injection site	X	
Jejunum	X	
Kidneys	X*	
Lachrymal gland		
Larynx	X	
Liver	X*	
Lungs	X	
Lymph nodes, cervical		
Lymph nodes mandibular	X	
Lymph nodes, mesenteric	X	
Mammary Gland	X	
Nasal cavity		
Optic nerves	X	
Ovaries	X*	
Pancreas	X	
Parathyroid	X	
Peripheral nerve		
Pharynx		
Pituitary	X*	
Prostate	X*	
Rectum	X	
Salivary gland	X	
Sciatic nerve	X	
Seminal vesicles	X	
Skeletal muscle	X	
Skin	X	
Spinal cord	X	
Spleen	X*	
Sternum		
Stomach	X	
Testes	X*	
Thymus	X	
Thyroid	X*	
Tongue	X	
Trachea	X	
Urinary bladder	X	
Uterus	X	
Vagina	X	
Zymbal gland		

X, histopathology performed
 *, organ weight obtained

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary

In the previous submission Serial #077 dated May 19, 2005, the draft report of study (b) (4)-446005 was submitted to support the change in administration of DX-88 in clinical trial DX-88/5 EDEMA2 from intravenous to subcutaneous. According to the phone conversation with Dr. John Lee, the clinical reviewer of the Serial #077, there were no holding issues regarding the route change from intravenous to subcutaneous for submission Serial #077. Based on the meeting package from the sponsor in Serial #100, the clinical trial amendment 5 submitted in Serial #077 was conducted in patients. Among these patients, 58 out of 240 attacks were treated with subcutaneous dosing.

In the submission S113, sponsor provided a table which listed editorial changes from the draft report submitted in Serial #077 to the final report submitted in Serial #090. Therefore, there were no significant changes made from draft report to final report, which would have impact on the conclusion.

In this single dose mini-pig toxicity study, the NOAEL was not identified. The immediate toxicity was not determined due to the lack of earlier sacrifice for gross and histopathology evaluation. The PK data showed 15 mg/kg sc resulted in approximately 1.3-to 1.5 fold higher AUC, but approximately 3-4 fold lower C_{max} than the i.v. administration at the same dose. The toxic profile for s.c. and i.v. doses at 15 mg/kg seemed comparable in terms of the finding of lung edema.

Recommendation: No action indicated this time.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

Linked Applications

Sponsor Name

Drug Name

IND 10426

DYAX CORP

Kallikrein Plasma Inhibitor (recombinant,
Pichia pastoris, Avecia Biotechnology)

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/s/

JEAN WU

07/18/2006

Non-Clinical Reviewer

CHING-LONG J SUN

07/25/2006

Non-Clinical Reviewer

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: BB-IND# 10426

Review number: 00

Sequence number/date/type of submission: S098/January 17, 2006/ IND Amendment S106/April 5, 2006/Response to information request

Information to sponsor: Yes () No ()

Sponsor and/or agent:

Dyax Corp.
300 Technology Square
Cambridge, MA 02139

Manufacturer for drug substance:

Reviewer name: Jean Q. Wu

Division name: Division of Pulmonary and Allergy Products

HFD #: 570

Review completion date: April 25, 2006

Drug:

Trade name: N/A

Generic name: Recombinant Human Plasma Kallikrein Inhibitor

Code name: DX-88

Chemical name: N/A

Relevant INDs/NDAs/DMFs: BB-IND#

(b)
(4)

Drug class: Biologics

Intended clinical population: Hereditary Angioedema Patients

Clinical formulation:

Liquid DX-88: sterile preservative-free liquid, 2-mL vial of active drug 1.1-1.2 mL of 10 mg/mL of DX-88 in PBS, pH 7.0. Placebo: 1 mL of PBS, pH 7.0

Lyophilized DX-88: sterile powder cake comprised of 30 mg DX-88 in 10 mM histidine in 10% sucrose, pH 6.5. Placebo: 10 mM histidine in 10% sucrose, pH 6.5; for administration, lyophilized drug and placebo will be reconstituted with 1.0 mL of water for injection

Route of administration: Subcutaneous

Proposed clinical protocol: A phase I randomized, double-blinded, crossover study was proposed to assess the bioequivalence and safety profiles of 30 mg DX-88 liquid versus lyophilized formulations in healthy volunteers. DX-88 liquid 30 mg will be dosed once via 3 X 1 mL s.c injections (1 mL each on arm, thigh and abdomen) plus 1 mL

lyophilized placebo. DX-88 lyophilized 30 mg will be dosed once with 1 mL injection (on arm or thigh or abdomen) plus liquid placebo 3 X 1 mL (1 mL each on arm, thigh and abdomen).

Previous clinical experience: 8 clinical studies have been conducted. Each of these studies utilized a liquid formulation of DX-88 in PBS. The clinical information was obtained from this submission and the Attachment 1, section 2. Human Clinical Trial in the submission serial #100 (meeting package).

Completed clinical trials:

In Phase I clinical study, DX-88/1, single iv. dose of 10-80 mg was administered in healthy subjects for PK evaluation. There were no significant clinical changes in vital signs, ECG, hematology, clinical chemistry, urinalysis and skin bleeding times reported.

In the second Phase I clinical study, DX-88/6, 20 mg/m² DX-88 was administered with 10-minute iv infusion in 3 sequential doses one week apart and a 4th dose of 20 mg/m² was administered with 4-hour iv infusion.

In the 3rd Phase I study, DX-88/13, PK and safety were evaluated for 14-day repeat dose of DX-88 at 30 mg iv or 10 and 30 mg sc in healthy subjects. DX-88 bioavailability at 30 mg dose was comparable following iv and sc. administration. Dysmenorrhea in one subject at 10 mg and vaginal infection in one subject at 10 mg were listed as adverse event (AE).

In a Phase II trial (DX-88/2) completed in the EU, safety, efficacy and PK of ascending doses of DX-88 (10, 40 or 80 mg iv.) were assessed in HAE patients. The AE reported breast mass NOS (not otherwise specified) in one out of 3 subjects at 40 mg dose and breast pain in one out of 3 subjects at 40 mg dose.

In a Phase II trial (DX-88/4), safety, efficacy and PK of ascending doses of DX-88 (5, 10, 20 or 40 mg/m²) were assessed in HAE patients.

On-going clinical trials:

In the Phase II study, EDEMA2 (DX-88/5), the administration of liquid DX-88 iv to sc. As of Jan. 10, 2006, 60 acute attacks of HAE have been used with liquid DX-88 sc. The current dose of DX-88 sc is 30 mg which requires three 1-mL injections of the current formulation of liquid DX-88 at 10 mg/mL. AE reported one menorrhagia of one subject at 20 mg/m².

In the Phase II study (DX-88/14), safety and efficacy of DX-88 (30 mg sc, three 1- mL injections) is assessed for the treatment of acute attacks of angioedema in HAE patients.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: Study No. (b).-446012

Study title: A Toxicokinetic and Injection Site Reaction Study of DX-88 Administered Subcutaneously as Liquid and Lyophilized Formulations in Mini-pigs. (Audited Draft Report)

Studies not reviewed within this submission: None

TABLE OF CONTENTS

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW.....	1
2.6.1 INTRODUCTION AND DRUG HISTORY.....	1
2.6.2 PHARMACOLOGY.....	5
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	5
2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....	5
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	5
2.6.6 TOXICOLOGY.....	5
2.6.6.1 Overall toxicology summary	Error! Bookmark not defined.
2.6.6.3 Repeat-dose toxicity:	5
2.6.7 TOXICOLOGY TABULATED SUMMARY	10
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	10

2.6.2 PHARMACOLOGY

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity:

Study title: A Toxicokinetic and Injection Site Reaction Study of DX-88 Administered Subcutaneously as Liquid and Lyophilized Formulations in Mini-Pigs. (Study No. (b) 446012)

Key study findings:

Mini pigs were dosed with DX-88 liquid form and DX-88 lyophilized form at 15 mg/kg (once on Day 0) and 1.5 mg/kg on Days 4, 7, 10 and 13.

Transient increase in APTT in both treated groups was consistent with its pharmacological action and not considered an adverse effect.

Lower level of phosphorus in both treated group males on Day 0, resolved on Day 1. For DX-88 lyophilized group males, the values maintained comparable to the control from Day 1 through Day 14. The value was lower on Day 14 for DX-88 liquid group males.

Gross and histopathologic findings of abscess/inflammation in mammary glands in 2 males and 1 female treated with DX-88 lyophilized form were considered incidental.

No local toxicity of injection sites was found in histopathologic evaluation.

Histopathology data for other collected tissues are to be completed.

No individual PK data and complete PK analysis. The summary of PK data showed that the PK profile in formulation of DX-88 lyophilized was comparable to DX-88 liquid formulation.

Study no.: (b) -4460012

Volume #, and page #: Vol. 2-3. Pages 1-568

Conducting laboratory and location:

(b) (4)

Date of study initiation: October 13, 2005

GLP compliance: GLP (audited draft, no signed GLP compliance statement)

QA report: yes () no (x)

Drug, lot #, and % purity: DX-88 liquid, Lot No. 227-01-003, purity: 99.51%

DX-88 lyophilized: Lot No. 05RP029, purity: 99.6%

Methods

Hanford mini-pigs (approximately 3 months old with body weight ranging from 12.2 to 16.4 kg for males and 11.5 to 17.5 kg for females at initiation of dosing) were administered subcutaneously as listed in the tables below. The dosing area was the inner thigh in the hindlimb. Formulated DX-88 liquid or lyophilized or vehicle (10 mM histidine, 10% sucrose, pH 6.5) were injected as a slow sc. bolus.

Study Day 0 Administration:

Group Number	Test Article ^a	Dosage Level ^b (mg/kg)	Label Concentration (mg/mL)	Actual Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
						Males	Females
1	Vehicle	0	0	(b) (4)	0.5	4	4
2	DX-88 Liquid	15	10	(b) (4)	1.5	4	4
3	DX-88 Lyophilized	15	30	(b) (4)	0.5	4	4

^a = For doses administered on study day 0, the total dose volume for each animal was divided into 2 equal portions and each portion was injected into a separate dose site (a total of 2 injection sites/animal on study day 0).

^b = The dosage levels selected for administration on study day 0 was 15 mg/kg for DX-88 liquid and lyophilized based on the theoretical dose concentrations of 10 mg/mL for DX-88 liquid and 30 mg/mL for DX-88 lyophilized. However, based on the actual test article concentrations the DX-88 liquid dose level was 13.7 mg/kg and the DX-88 lyophilized dose level was 12.9 mg/kg.

Study Days 4, 7, 10 and 13 Administration:

Group Number	Test Article ^a	Dosage Level ^b (mg/kg/dose)	Label Concentration (mg/mL)	Actual Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals ^c	
						Males	Females
1	Vehicle	0	0	(b) (4)	0.05	4	4
2	DX-88 Liquid	1.5	10	(b) (4)	0.15	4	4
3	DX-88 Lyophilized	1.5	30	(b) (4)	0.05	4	4

^a = Doses administered on study days 4, 7, 10 and 13 were injected into a separate injection site designated for each of the dosing days (a total of 4 separate injection sites/animal over these dosing days).

^b = The dosage levels selected for administration on study days 4, 7, 10 and 13 was 1.5 mg/kg/dose for DX-88 liquid and lyophilized based on the theoretical dosage concentrations of 10 mg/mL for DX-88 liquid and 30 mg/mL for DX-88 lyophilized. However, based on the actual test article concentrations the DX-88 liquid dosage level was 1.4 mg/kg/dose and the DX-88 lyophilized dosage level was 1.3 mg/kg/dose.

^c = Same animals from study day 0.

Observation and Times:

Mortality and moribundity were assessed twice daily, and clinical observations were performed prior to dose, 1, 2, 4 and 8 hours post-dose, and daily on non-dosing days. Detailed physical examinations and body weight were recorded weekly.

Hematology, clinical chemistry and urinalysis were evaluated prior to the initiation of dosing (Day -3), 2 and 24 hours post dose on study day 0, and prior to the scheduled necropsy (Day 14). Activated partial thromboplastin time (APTT) was evaluated at 0 (pre-dose), and 0.25, 0.5, 1, 4, 8, 12, 18 and 24 hours post dose on study day 0.

Complete necropsy was performed on all animals. As listed in the inventory table at the end of section 2.6., selected organs were weighed and selected tissues and oviducts from all group animals were examined microscopically (without peer review).

Blood samples for TK evaluation were collected at 0 (pre-dose), 0.25, 0.5, 1, 4, 8, 12, 18 and 24 hours post dose on study day 0.

Results:

Mortality: None.

Clinical signs: There were no test article-related clinical observations reported.

Body weights: The cumulative body weights in both female treated groups were higher than the control group during 0-2 week period. The similar trend was not observed in males.

Hematology: APTT (activated partial thromboplastin time) was prolonged in all male and female treated groups. The data was listed in the table below. APTT prolongation occurred 15 min to 2 hours post-dose in all treated groups. Mean APTT returned to comparable control values by 12 hours post-dose in affected males, by 18 hours post-dose in 15 mg/kg liquid DX-88 group females and by 8 hours post-dose in 15 mg/kg lyophilized DX-88 group females.

The transient prolongation in APTT was consistent with the pharmacologic effect of DX-88 involving kallikerin inhibition (Williams and Baird, 2003, Ritchie, 2003), hence, was not considered as an adverse effect.

Text Table 1: Mean Activated Partial Thromboplastin Times Following DX-88 Liquid or Lyophilized Administration Presented as : Seconds (% from control)

Dose Group (mg/kg)	Males			Females		
	0	15 LIQ	15 LYO	0	15 LIQ	15 LYO
Study Day 0						
Pre-dose	16.8	16.6(-1.2)	16.8(0.0)	16.9	17.6(4.1)	16.9(0.0)
0.25 hour post-dosing	17.4	20.9(20.1)	20.1(15.5)	16.5	21.7(31.5)	22.5*(36.4)
0.5 hour post-dosing	17.7	25.6(44.6)	24.5(38.4)	16.4	23.9(45.7)	25.9*(57.9)
1 hour post-dosing	18.0	27.1**(50.6)	24.9**(38.3)	16.5	25.9**(57.0)	26.8**(62.4)
2 hours post-dosing	17.2	30.5**(77.3)	28.5**(65.7)	16.7	29.7**(77.8)	30.6**(83.2)
3 hours post-dosing	18.0	29.4**(63.3)	28.6**(58.9)	17.0	29.9**(75.9)	25.0**(47.1)
4 hours post-dosing	16.5	27.5**(66.7)	26.3**(59.4)	16.5	29.0**(75.8)	25.0**(51.5)
8 hours post-dosing	16.6	20.4**(22.9)	19.5*(17.5)	16.0	22.5**(40.6)	18.4(15.0)
12 hours post-dosing	16.8	16.7(-0.6)	17.9(6.5)	15.7	18.2**(15.9)	17.0(8.3)
18 hours post-dosing	16.4	16.0(-2.4)	16.5(0.6)	15.6	16.7(7.1)	16.6(6.4)
24 hours post-dosing	16.9	16.5(-2.4)	17.0(0.6)	15.4	16.9(9.7)	16.6(7.8)

* = Statistically significant compared to the control group at p<0.05 using Dunnett's test.

** = Statistically significant compared to the control group at p<0.01 using Dunnett's test.

Clinical Chemistry: Mean phosphorus values in both test article treated group males were lower than the control group post Day 0 administration. The reduction was resolved by study day 1 in both affected group males and remained comparable to the control group values on study day 14 in DX-88 lyophilized group males. The lower phosphorus

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level was observed on study day 14 in DX-88 liquid group males. No other test article-related alterations were observed.

Urinalysis: No test article-related alterations were reported. The urine potassium values in female DX-88 treated groups were higher than the respective control group values. However, due to the small sample size (n=2) for each group of females and the large variations of this measurement, the difference was more likely to be a biological variation than a test article-related effect.

Gross pathology: Masses in mammary glands were observed in 2 (animals 43690 and 43692) out of 4 males and 1 (animal 43700) out of 4 females treated with DX-88 lyophilized formulation, which were confirmed as abscesses in microscopic examination. A mass in mammary gland was also observed in 1 (animal 43699) out of 4 control females, which was confirmed as chronic active inflammation in microscopic examination.

In the sponsor's response submitted in S106, it stated that the masses consistent with those found postmortem were identified in the preclinical physical examination in animals 43690, 43699 and 43700 prior to treatment, indicating the finding was not related to the test article treatment. Based on the information in S106, the finding of abscessation/inflammation was very likely a common observation in swine instead of a test article related effect. Therefore, the similar finding in one male (No. 43692) observed postmortem but not prior to the treatment was considered incidental.

Organ weights: There were no reported test article-related organ weight changes.

Histopathology: No local toxicity was found at injection sites.

Two of 4 males and 1 out of 4 females treated with DX-88 lyophilized formulation showed abscess in mammary gland and 1 control female showed active chronic inflammation in mammary gland. Based on the sponsor's response in S106, the finding of abscess in mammary gland was considered incidental (see the section of Gross Pathology result above).

The histopathology evaluation for all collected tissues was not completed yet.

Toxicokinetics: Only preliminary PK analysis was provided. No individual data and PK analysis were submitted. Based on the summarized PK table (shown below) and figure, the PK profile of sc dose in formulation of DX-88 lyophilized was comparable to that in DX-88 liquid formulation.

Table 2: Pharmacokinetic Parameters for Liquid and Lyophilized DX-88 to Male and Female Mini Pigs (15 mg/kg, sc).

Pharmacokinetic Parameter	Calculated Values	
	Liquid	Lyophilized
Formulation		
$t_{1/2}$ (hr)	2.08 ± 0.01	2.34 ± 0.50
Cl (mL/min/kg)	3.40 ± 0.73	4.15 ± 0.94
Vd (mL/kg)	624 ± 204	828 ± 194
C_{max} (µg/mL)	13.5 ± 4.67	12.3 ± 2.84
t_{max} (hr)	3.00 ± 0.82	2.03 ± 1.00
AUC _{0-∞} (µg x hr/mL)	77.1 ± 20.0	62.5 ± 11.3

Values represent mean ± SD

Abbreviations: $t_{1/2}$ = elimination half-life; Cl = clearance; Vd = apparent volume of distribution; C_{max} = maximal DX-88 concentration; t_{max} = time after dosing C_{max} observed; AUC_{0-∞} = area under concentration versus time curve.

Histopathology inventory (optional)

Study	single dose		
Species	Mini-pig		
Adrenals	X*		
Aorta	X		
Bone Marrow smear	X		
Bone (femur)	X		
Brain	X*		
Cecum	X		
Cervix	X		
Colon	X		
Duodenum	X		
Epididymis	X*		
Esophagus	X		
Eye	X		
Fallopian tube			
Gall bladder	X		
Gross lesions	X		
Harderian gland			
Heart	X*		
Ileum	X		
Injection site	X		
Jejunum	X		
Kidneys	X*		
Lachrymal gland			
Larynx	X		
Liver	X*		
Lungs	X		
Lymph nodes, cervical			
Lymph nodes mandibular	X		

Lymph nodes, mesenteric	X			
Mammary Gland	X			
Nasal cavity				
Optic nerves	X			
Ovaries	X*			
Pancreas	X			
Parathyroid	X			
Peripheral nerve	X			
Pharynx				
Pituitary	X*			
Prostate	X*			
Rectum	X			
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X			
Skeletal muscle	X			
Skin	X			
Spinal cord	X			
Spleen	X*			
Sternum				
Stomach	X			
Testes	X*			
Thymus	X			
Thyroid	X*			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	X			
Vagina	X			
Zymbal gland				

X, histopathology performed

*, organ weight obtained

2.6.7 TOXICOLOGY TABULATED SUMMARY

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: Based on the draft report of this PK and local reaction study, the s.c. administration with DX-88 liquid form and DX-88 lyophilized form at 15 mg/kg (once on Day 0) and 1.5 mg/kg on Days 4, 7, 10 and 13 did not show localized toxicity at the injection sites. The transient increase in APTT was consistent with its pharmacological action and not considered an adverse effect. The test article-related effect on phosphorus in both treated group males on Day 0 may not be ruled out. However, the finding was resolved from Day 1 and only reoccurred on Day 14 for liquid DX-88 group males. It was not associated with clinical abnormalities and was not found in females. In addition, the phosphorus level is clinically monitorable, and hence, was not considered as a significant safety concern for the proposed clinical study. The preliminary PK analysis

showed summarized PK data in a table and a figure, which indicated that PK profile in the formulation of liquid DX-88 was comparable to the lyophilized DX-88 formulation.

Based on the further information submitted in S106, the finding of the mass in mammary gland observed in two males (animals 43690 and 43692) and one female (animal 43670) treated with lyophilized DX-88 formulation was considered incidental since the gross finding was also observed in the male (No. 43690) and the female (No. 43670) prior to treatment, and in one control female (animal 43699) prior to and post treatment. There was no clinical observation or clinical pathology findings associated with the finding of the abscessation/inflammation. No significant test article-related injection site reaction was observed. The histopathology evaluation for all collected tissues was not completed yet.

The proposed phase I study was to administer two doses of 30 mg DX-88 in liquid form or lyophilized form to healthy subjects with a week apart. The dose of 30 mg liquid DX-88 s.c. has been applied in the previous and on-going clinical trials. Based on the PK summary data, the dose of 30 mg/kg lyophilized DX-88 was considered comparable to liquid DX-88. Since the systemic exposure was comparable for both formulations, no significant different histopathologic findings were expected for the remaining collected tissues of the animals treated with two formulations. Based on the PK data and histophologic evaluation of the limited tissues, there was no significant safety concern for the proposed clinical trial.

Recommendation:

Based on review of the summary PK data, the system exposure of lyophilized DX-88 was considered comparable to liquid DX-88 which has been tested at 30 mg in the previous and on-going clinical studies. There was no significant safety concern for the proposed human clinical study of both formulations from the preclinical point of view. The sponsor should be informed to submit the final report of this study (Study Number (b) 4460012).

Letter to sponsor:

Submit the final report of the study (Study Number (b),-4460012) within 120 days of the submission. Indicate any changes made from the submitted draft report in the cover letter or in the introduction of the final report. Also indicate if no change was made.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

Linked Applications

Sponsor Name

Drug Name

IND 10426

DYAX CORP

Kallikrein Plasma Inhibitor (recombinant,
Pichia pastoris, Avecia Biotechnology)

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/s/

JEAN WU

04/25/2006

Non-Clinical Reviewer

CHING-LONG J SUN

04/26/2006

Non-Clinical Reviewer

concur.

Part C – Non-Clinical Pharmacology/Toxicology Reviewer(s)

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	Y <input checked="" type="radio"/> N	No section of 2.1 listed. However, it is an e-submission, which shows the overall table of contents by itself.
Introduction to the summary documents (1 page) [2.2]	<input checked="" type="radio"/> Y N	
Non-clinical overview [2.4]	<input checked="" type="radio"/> Y N	
Non-clinical summary [2.6]	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> Pharmacology	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> Pharmacokinetics	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> Toxicology	<input checked="" type="radio"/> Y N	

CTD Module 4 Contents	Present?	If not, justification, action & status
Module Table of Contents [4.1]	Y <input checked="" type="radio"/> N	No section of 4.1 listed. However, it is an e-submission, which shows the table of contents in Module 4 by itself.
Study Reports and related info. [4.2]	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> Pharmacology	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> Pharmacokinetics	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> Toxicology	<input checked="" type="radio"/> Y N	
Literature references and copies [4.3]	<input checked="" type="radio"/> Y N	

Examples of Filing Issues	Yes?	If not, justification, action & status
content, presentation, and organization sufficient to permit substantive review?	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> legible	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> English (or translated into English)	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> compatible file formats	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> navigable hyper-links	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> interpretable data tabulations (line listings) & graphical displays	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> summary reports reference the location of individual data and records	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> protocol-specified (as opposed to a different, post-hoc analysis) and other critical statistical analyses included	<input checked="" type="radio"/> Y N	
<input type="checkbox"/> all electronic submission components usable	<input checked="" type="radio"/> Y N	
data demonstrating comparability of product to be marketed to that used in clinical trials (when significant changes in manufacturing processes or facilities have occurred)	Y <input checked="" type="radio"/> N/A	Not applicable in this part. Refer to Part B and Part D for this item.
for each non-clinical laboratory study, either a statement that the study was conducted in compliance with the good laboratory practice requirements set forth in 21 CFR Part 58 or, if the study was not conducted in compliance with such	<input checked="" type="radio"/> Y* N	*Pivotal studies were conducted in GLP compliance. The non-GLP studies submitted are either pharmacodynamic assays, pharmacokinetics and distribution studies which are considered acceptable, or other supportive, exploratory or

