

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number **20-823**

PHARMACOLOGY REVIEW(S)

K1.5



K1.5

N20823



N20823

REC -
5/4/00
9:03 AM

ExelonTM **(Rivastigmine Tartrate)**

1.5 mg, 3.0 mg, 4.5 mg, & 6.0 mg

NDA 20-823

Pharmacology

PHARMACOLOGY

NDA 20-823

ExelonTM

(Rivastigmine Tartrate) Capsules

, 1.5 mg, 3.0 mg, 4.5 mg, & 6.0 mg

Classification: 1S

<u>Date</u>	<u>Document</u>	<u>Tab</u>
10/26/97	Pharmacology Review #1: B. Rosloff, Ph.D.	D
12/23/97	Executive CAC Committee Memo	E
2/18/98	Statistics CAC Review # 1: S. Wang, Ph.D.	F
3/4/98	Supervisory Review: G. Fitzgerald, Ph.D.	G
7/7/98	NOT APPROVABLE Letter to Firm	

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ON ORIGINAL**

Barry N. Rosloff, Ph D.
10/26/97

PHARMACOLOGIST REVIEW OF NDA 20-823

ORIGINAL SUMMARY

SPONSOR: Novartis Pharmaceuticals Corporation
59 Route 10
East Hanover, NJ 07936-1080

DRUG: ENA 713
e; Exelon™)

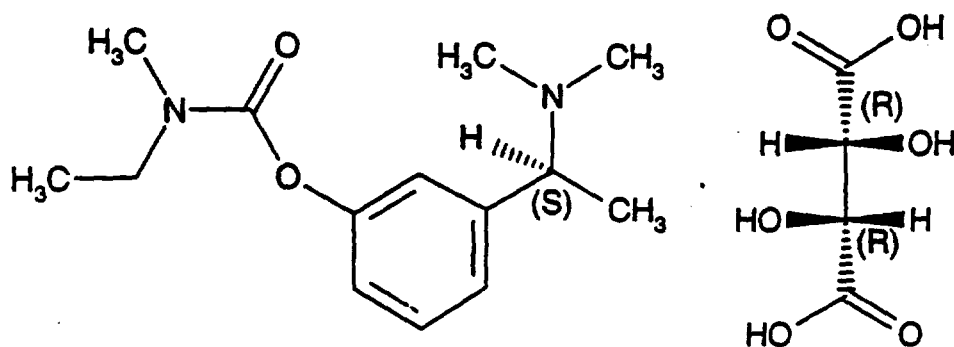
(Chemical structure, chemical name, code numbers, etc. shown on the 2 following pages).

CATEGORY: cholinesterase inhibitor for use in Alzheimers' Disease

RELATED IND: _____

LABS WHERE STUDIES WERE PERFORMED:

All pivotal toxicity, carcinogenicity, and reproduction studies were done at Sandoz Pharmaceuticals Corp., East Hanover, NJ, with the exception of 1 of the 2 rat segment II and III studies (the second of each pair listed in the Table of Contents, below) which were done at New _____, and the genotoxicity studies, which, except for the human lymphocyte study which was done at _____ were done at Sandoz Pharmaceuticals, Ltd., Basel, Switzerland.

ENA 713 Capsules**SECTION II: Summary of the CMC section****D CHEMISTRY, MANUFACTURING AND CONTROLS****1 DRUG SUBSTANCE (Cont.)****1.1 Description (Cont.)****1.1.2 Physical and Chemical Characteristics****Structural Formula****Molecular Formula****Molecular Weight**

$$250.3 + 150.1 = 400.4$$

Appearance

White or off-white, finely crystalline powder

ENA 713 Capsules

SECTION II Summary of the CMC section

D CHEMISTRY, MANUFACTURING AND CONTROLS

1 DRUG SUBSTANCE

1.1 Description

1.1.1 Names

Chemical Name

(S)-N-Ethyl-N-methyl-3-[1-(dimethylamino)ethyl]-phenyl carbamate,
hydrogen-(2R,3R)-tartrate

Generic Name

Chemical Abstracts Registry Number

129101-54-8

Laboratory Codes

212-713

212-713 hta

SDZ 212-713

SDZ 212-713 hta

ENA 713

ENA 713 hta

SDZ ENA 713

SDZ ENA 713 hta

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Sponsor's Pharmacodynamics summary	
Sponsor's ADME/PK summary	
Dr. DeGeorge's Review of _____	

PHAMACODYNAMICS

The sponsor's summary is attached. Much of this data has been previously reviewed in Dr. DeGeorge's review, attached. Selected points are further discussed in the Labeling section of the present review.

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ADME/PK

The sponsor's summary is attached. Several of the studies were previously reviewed in Dr. DeGeorge's review, attached. Selected points are further discussed in the Summary and Labeling sections of the present review.

**APPEARS THIS WAY
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ONE YEAR P.O. TOXICITY IN RATS:

A) Dosage

25/sex at 0, 0.2, 0.6, 1.8, or 3.0 mg/kg/day, by gavage.

(Doses expressed as hydrogen tartrate salt. Doses expressed as free base = 0.1, 0.4, 1.1, and 1.8 mg/kg, resp.)

Of the above animals, 5/sex/group were kept for a 2 month recovery period.

Strain: _____

Drug lot# : 90904

B) Results

1) Observed signs

The following were seen, primarily at the 2 highest dose groups, and generally D-R:

- a) Tremors/twitches/flutterers
- b) Decreased locomotor activity (+ a low incidence of increased activity in F).
- c) Chomping/chewing/licking/cage biting/gnawing at fingers/excessive grooming
- d) Salivation
- e) Ataxia
- f) Flattened body position
- g) Urogenital staining, wet/damp fur
- h) Exophthalmos (F only)
- i) Labored/rapid breathing, loose stool/diarrhea. (HD M only)

Some of the above were also seen, mostly with relatively low incidence, at the 2 lower doses.

(Primarily "a" and "c", above). An incidence table was shown for the recovery period in which some of the above signs were seen (primarily "a", "c", "d", "f", and "h", above); it cannot be determined from the data presented if the incidence was decreasing over the recovery period, although some reversibility can be inferred since most of these signs were not seen at doses at which they were seen during treatment).

2) Mortality

No drug effect.

The following numbers of deaths/moribund sacrifices occurred during the treatment period:

	<u>C</u>	<u>LD</u>	<u>MD</u>	<u>M-HD</u>	<u>HD</u>
M	8	3	5	2	4
F	4	3	1	2	1

An additional control M and M-HD M died during the recovery period.

3) Bodyweight gain

Decreased at M-HD and HD. Mean weights at 1 year approx. 90%, 85%, 80 %, and 75% of control in M-HD M, M-HD F, HD M, and HD F, resp.

Weight gain was increased in LD F (mean~10- 15% above control near end of study) and to a lesser degree in MD F and (not statistically significant) in MD M.

4) Food Consumption

Decreased in M-HD M, HD M, and HD F. (Decreases seen in M-HD F during first few weeks only.) Increased in LD F and to lesser degree in MD F.

5) Ophthalmoscopic exam

(Done pre-study and weeks 15, 28, 51, 59).

No drug effect.

6) Hematology

(Done in 10/sex/group at weeks 4, 8, 13, 26, 39, and 52, and in surviving recovery animals after recovery period).

No drug effects.

Parameters measured: RBC, Hb, Hct, platelets, reticulocytes, nucleated RBC, WBC, differential.

7) Blood chemistry

(Done in same animals as hematology, above).

a) Triglycerides decreased, primarily in M-HD F and HD F at most time points. (Mean ~ 1/3 - 2/3 control). (Not apparent after recovery period). Sporadic decreases seen in lower dose F groups and in HD M.

b) Cholesterol slightly increased in HD F at most times (but not after recovery period); sporadic slight increases also in lower dose F groups.

c) One M-HD M had, at end of recovery period only, elevated ALT, AST, AP, total bilirubin, and urea nitrogen, and decreased total protein. (This animal died during recovery period with multifocal reticulum cell sarcoma, considered to be unrelated to treatment).

d) Other parameters measured: glucose, Na, K, Cl, plasma and RBC cholinesterase.

8) Urinalysis

(Done in same animals as hematology and blood chemistry, above).

HD M had a marginally increased frequency of the maximum score for urinary protein at weeks 8, 13, and 26; thereafter most animals in all groups, including controls, had the maximum score.

9) Organ weights

No drug effects, aside from differences attributable to effects on bodyweight.

10) Gross pathology

No drug effects.

11) Microscopic pathology

(Routine exam. done in all animals).

No clear drug effects.

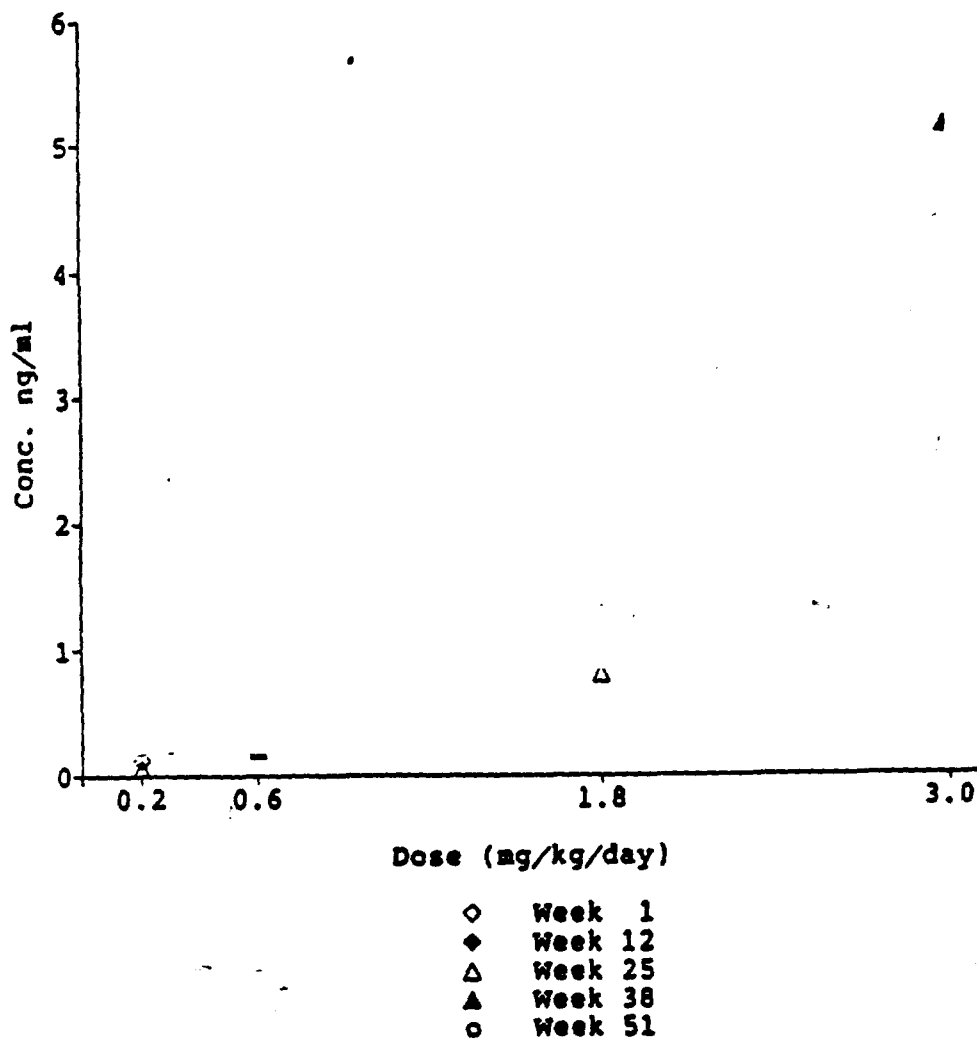
The incidence of bile duct hyperplasia among non-recovery animals was 1/40, 3/40, 4/40, 3/40, and 7/40 in control, LD, MD, M-HD, and HD, resp. (All in males except for one female in each of the drug-treated groups). This was considered to be spontaneous according to the text. The incidence among recovery animals was 1/10, 0/10, 0/10, 0/10, and 1/10 in C, LD, MD, M-HD, and HD, resp. (Both in males).

12) Toxicokinetics

Sampling was from 5/sex/group, 1 hour post-dose during weeks 1, 12, 25, 38, and 51. Blood was analyzed for parent compound and metabolite ZNS 114-666. Limit of quantitation was . Sample tubes contained physostigmine to inhibit hydrolysis of the parent compound.

Sponsor's summary graphs are attached. Levels of parent compound were generally low, being near or below the limit of quantitation in most animals at the 2 lower doses. Levels of the metabolite were much higher. Levels of both compounds increased roughly in proportion to dose, and showed a tendency to be slightly lower at week 1 than at later times. Results were not broken down by sex.

ONE YEAR ORAL TOXICITY STUDY IN THE RAT
BLOOD CONCENTRATIONS OF SDZ ENA 713 at T=1h



ONE YEAR ORAL TOXICITY STUDY IN THE RAT
BLOOD CONCENTRATIONS of ZNS 114-666 at T 1h

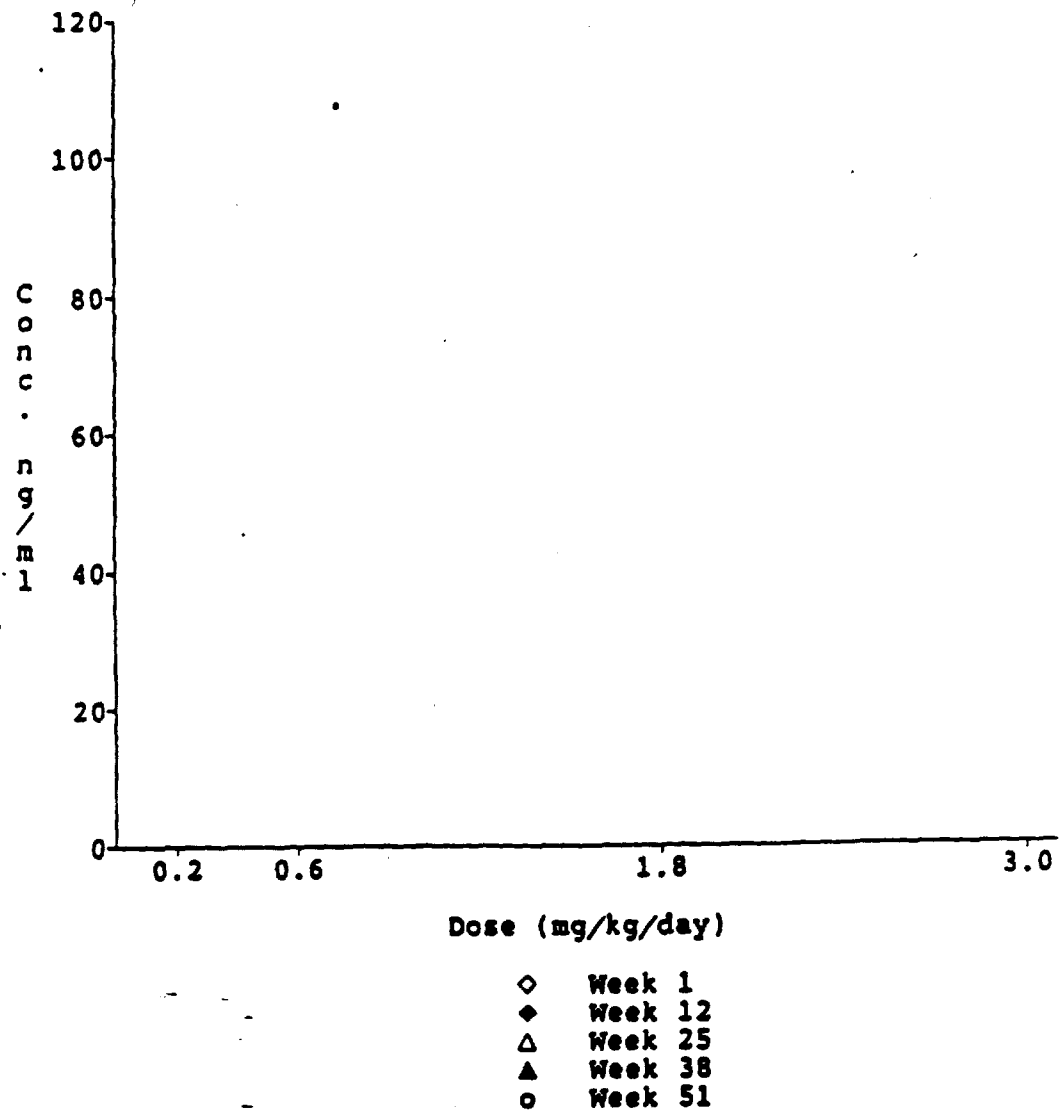
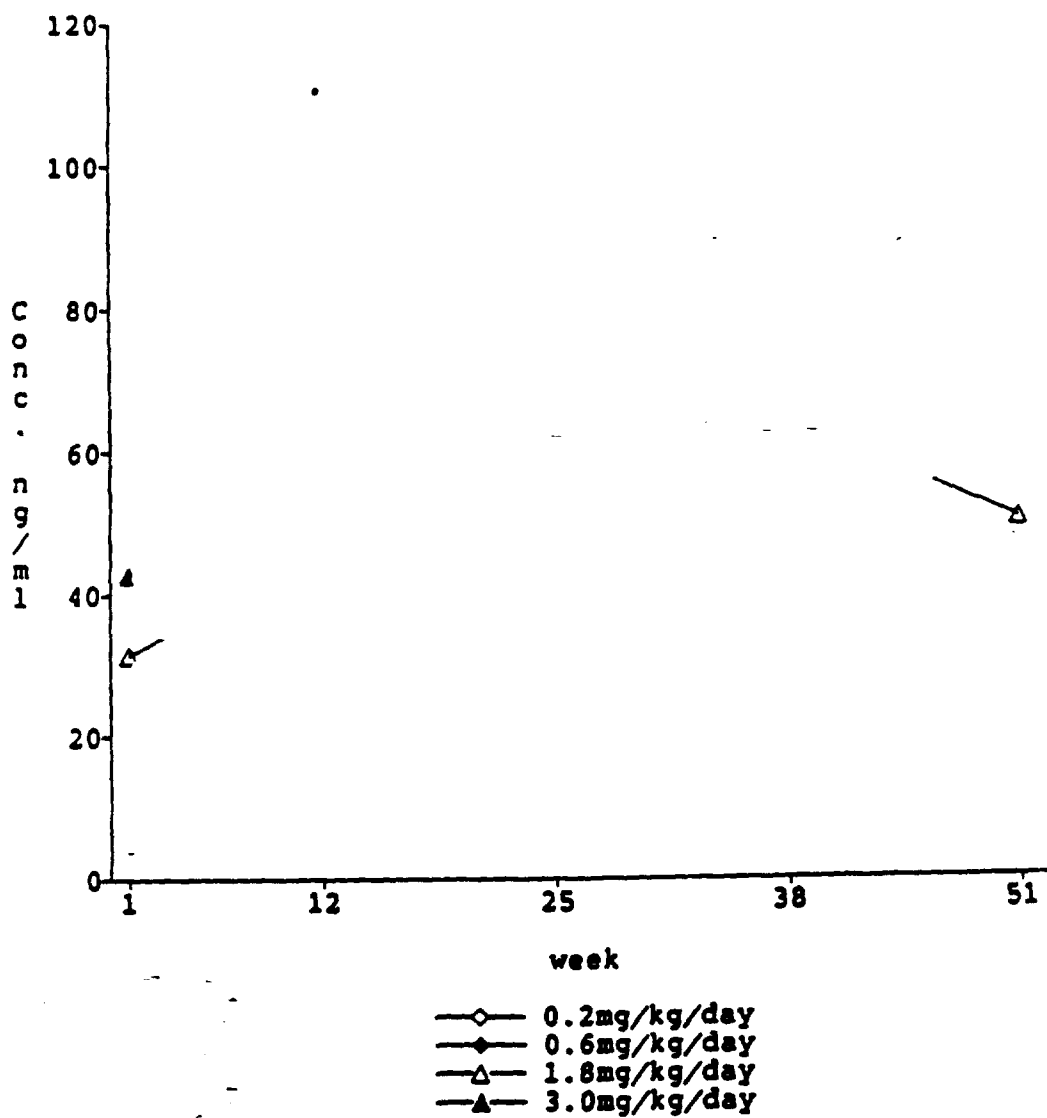


Figure 3

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ONE YEAR ORAL TOXICITY STUDY IN THE RAT
BLOOD CONCENTRATIONS of ZNS 114-666 at T 1h



2 YEAR CARCINOGENICITY IN RATS:

A) Dosage

75/sex at 0, 0, 0.2, 0.6, or 1.8 mg/kg/day, by gavage.

(Doses expressed as hydrogen tartrate salt. Doses expressed as free base = 0.1, 0.4, and 1.1 mg/kg, resp.)

Strain:

Drug lot # 90904

B) Results

1) Observed signs

The text and summary table were not always in agreement (and no individual animal data were presented); the major effects appear to have been tremors, "twitches/flutter" salivation, changes (both increases and decreases) in locomotor activity, flattened body posture, "chomping/chewing", licking, gnawing on forelimb paws, excessive grooming, and wet fur. Most of the above were seen at MD and especially HD; effects at LD were generally absent/borderline/minimal. According to the text, the incidence of signs increased, and signs became evident at lower doses, over the course of the study.

There was no drug effect on the incidence of palpable masses.

2) Mortality

No drug effect.

Numbers (%) alive at termination as follows:

	<u>Control 1</u>	<u>Control 2</u>	<u>LD</u>	<u>MD</u>	<u>HD</u>
M	16 (21%)	10 (13%)	16 (21%)	15 (20%)	13 (17%)
F	24 (32%)	18 (24%)	22 (29%)	19 (25%)	21 (28%)

3) Bodyweight gain

Decreased at HD

Weights near end of study at HD were approx. 90 % and 80-85% of control in M and F, resp. A slight, NS decrease was also seen in LD and MD F during the second half of the study (means generally > 95% of control). Bodyweight curves are attached.

4) Food consumption

Decreased at HD; means 5-10 % below control. Smaller, generally NS decreases seen in MD F.

5) Ophthalmoscopic exam

(Done pre-study and, in left eye only, weeks 26, 52, 78, and 103).

Text states no drug effect, although it is noted that the summary table presented is incomprehensible (e.g. denominators of 75 are given for all findings; there is no indication of how this should be broken down by treatment week [including pre-treatment results!])

6) Organ weights

Relative adrenal weight slightly increased at HD (1.3-1.4 x); absolute adrenal weight slightly increased but NS.

7) Gross pathology

Increased incidence of hind limb skin ulcers in HD M (21/75 vs 12/150 in controls).
(See below)

8) Histopathology

(Routine exam done in all animals)

a) No drug-related increase in neoplasms

b) Increased incidence of hind limb pododermatitis/ulceration in HD M (area not routinely examined histologically; number of affected animals = 8, 5, and 23 in control-1, control -2, and HD, resp.). It was stated that "lesions of this type . . . are common in large male rats on wire caging, and the increased incidence . . . was felt to be incidental."

c) Increased incidence of hypercellularity of sternal bone marrow in MD and HD M (20/75, 18/75, 30/76, and 33/75 in control - 1, control-2, MD, and HD, resp.). It was stated that this was a response to the above-mentioned hind-limb inflammation; however note that although there was some association in the occurrence of these 2 lesions, the bone marrow finding was seen in more animals than was the hind-limb inflammation; also the former but not the latter was increased in MD M.

d) Increased incidence of erosion in stomach fundus and pylorus in HD M:

	<u>Control - 1</u>	<u>Control - 2</u>	<u>HD</u>
FUNDUS	3/75	3/75	11/75
PYLORUS	2/75	3/75	8/75
EITHER	4/75	5/75	16/75

e) Incidence of cysts in adrenal cortex in HD F was 11/74, vs 2/75 and 2/74 in the control groups. Said to be a common degenerative lesion in aging rats and considered to be unrelated to drug.

f) Incidence of plasmacytosis in sublumbar lymph node in HD M was 48/74, vs 27/73 and 30/73 in control groups. Not discussed in report; presumably considered incidental.

9) Toxicokinetics

Blood was sampled from 5/sex at "approximately" 1 hour post-dose during weeks 1, 13, 35, 70, and (in F only) 104. Sample tubes contained physostigmine to inhibit hydrolysis of parent compound. Samples were assayed for parent compound and the phenolic metabolite ZNS 114-666. Limit of quantitation

Summary table of results is attached. Levels of parent compound were very low. (Undetectable in most LD and MD rats; not shown in table). Levels of the metabolite were much greater (at HD, ~ 20-200x parent), and increased roughly in proportion to dose. It was stated that "little or no accumulation" of parent or metabolite occurred with increasing duration of treatment, although as indicated in the table mean values at HD did tend to increase. There were no obvious sex differences.

Figure AM-1

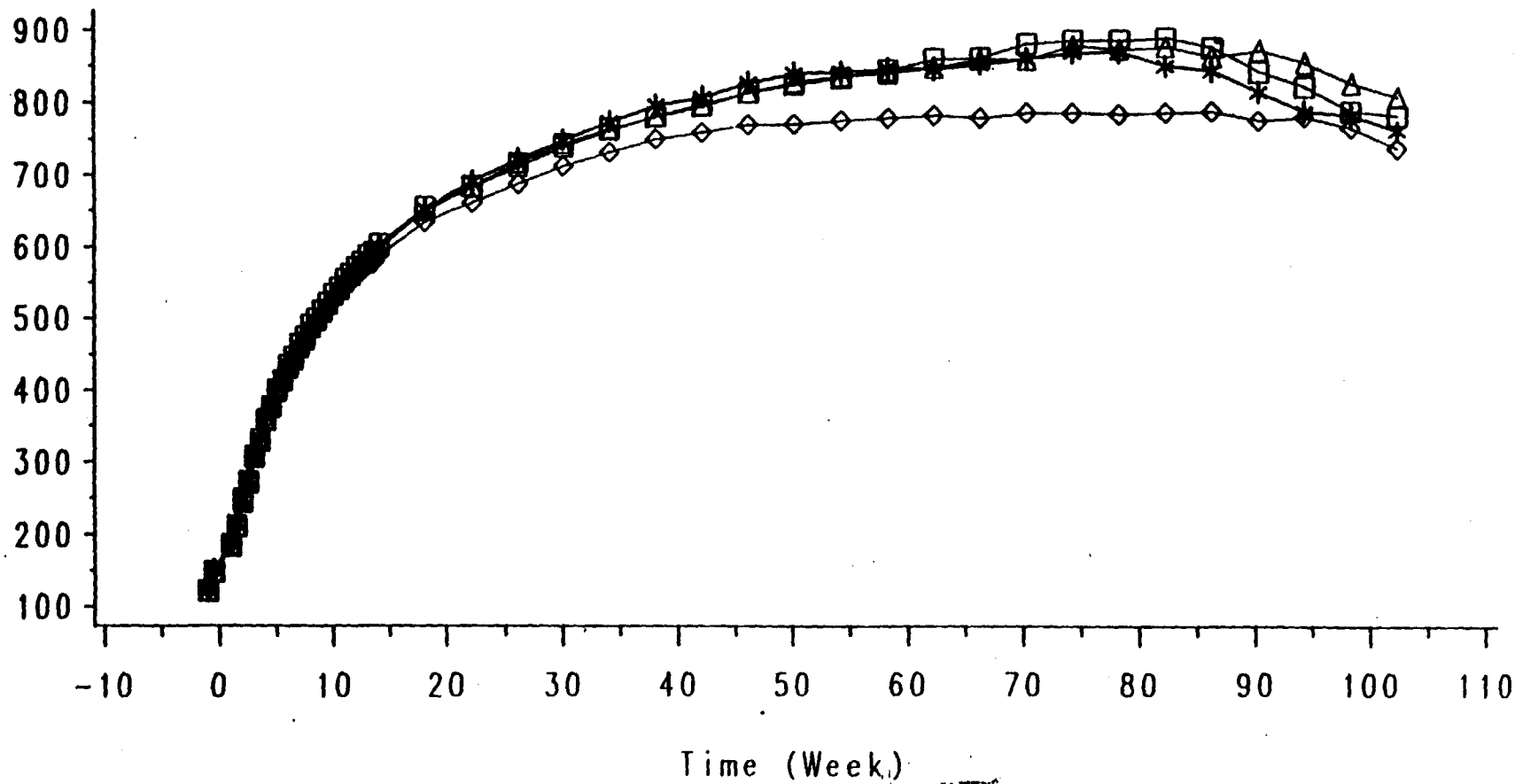
TWO-YEAR ORAL (GAVAGE) CARCINOGENICITY STUDY IN THE RAT
ON ENA 713

Sandoz Project T-2740

SEX=Male

Body Weight Means

Mean (GM)



page 10a

GROUP $\triangle-\triangle-\triangle$ CONTROL I + II $\square-\square-\square$ LOW DOSE
 $*-*-*$ MID DOSE $\diamond-\diamond-\diamond$ HIGH DOSE

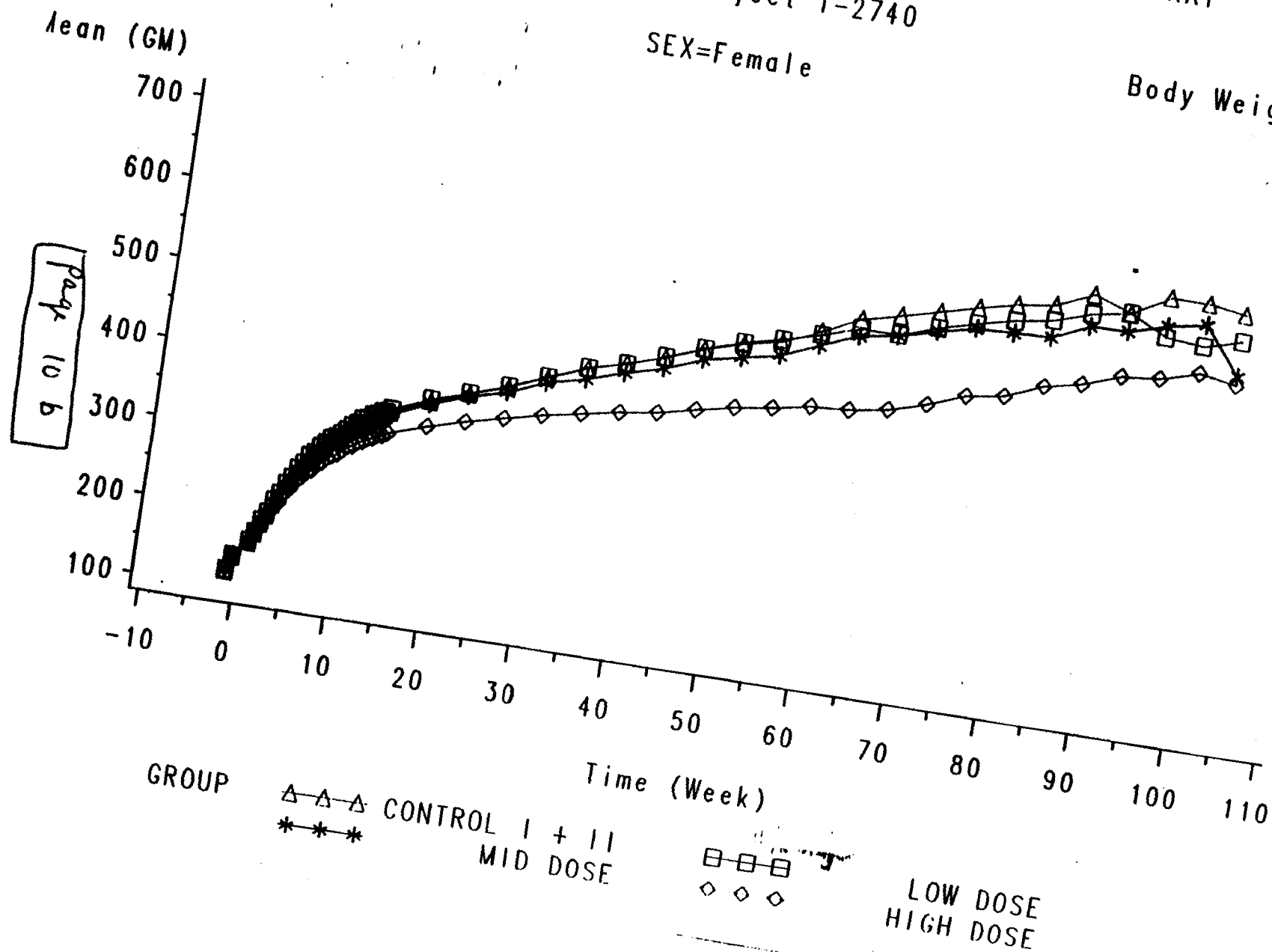
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Figure AM-1

TWO-YEAR ORAL (GAVAGE) CARCINOGENICITY STUDY IN THE RAT
ON ENA 713
Sandoz Project T-2740

SEX=Female

Body Weight Means



DM-1-2/18/93

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RAT CA - ~~1000~~ BLOOD LEVELS

TABLE 1						
BLOOD CONCENTRATIONS (1 h postdose) OF SDZ ENA 713 AND ITS PHENOLIC METABOLITE ZNS 114-666 IN THE RAT (PROJECT T-2740)						
Drug Week	Blood Concentration (ng/mL) ¹					
	0.12 mg/kg/day		0.36 mg/kg/day		1.09 mg/kg/day	
	M	F	M	F	M	F
SDZ ENA 713						
1	0 ± 0	0 ± 0	0.18 ± 0.20 ²	0.07 ± 0.14 ³	0.29 ± 0.22	0.29 ± 0.51
13	0 ± 0	0.06 ± 0.14	0.11 ± 0.27 ²	0.10 ± 0.21 ³	0.21 ± 0.21	0.69 ± 0.29
35	0.14 ± 0.32	0.05 ± 0.12	0 ± 0 ²	0 ± 0 ³	0.26 ± 0.15	0.97 ± 0.66
70	0 ± 0	0 ± 0	0.12 ± 0.20 ²	0 ± 0 ³	2.57 ± 2.09	0.86 ± 0.54
104	- ⁴	0.46 ± 0.63	-	0.49 ± 0.38	-	2.56 ± 2.29
ZNS 114-666						
1	3.99 ± 0.28	5.34 ± 0.54	28.05 ± 11.10 ²	23.11 ± 12.85 ³	45.64 ± 16.46	36.74 ± 4.91
13	5.18 ± 0.59	7.61 ± 2.01	17.07 ± 4.31 ²	17.30 ± 3.68 ³	36.50 ± 8.93	52.83 ± 15.60
35	4.61 ± 0.55	6.98 ± 0.95	17.35 ± 2.25 ²	20.58 ± 2.96 ³	51.93 ± 10.54	61.96 ± 7.49
70	7.32 ± 4.38	6.39 ± 1.15	21.04 ± 7.68 ²	18.31 ± 1.49 ³	72.92 ± 17.64	50.78 ± 8.82
104	-	6.56 ± 1.78	-	21.95 ± 5.18	-	50.61 ± 13.21

¹Mean ± SD, n=5²n=6³n=4⁴not determined

2 YEAR CARCINOGENICITY IN MICE:

A) Dosage

70/sex at 0, 0, 0.4, 1.0, or 2.5 mg/kg/day, by gavage.

(Doses expressed as hydrogen tartrate salt. Doses expressed as free base = 0.25, 0.6, 1.6 mg/kg, resp.)

Strain: _____

Drug lot# 90904

B) Results

1) Observed signs

a) LD - no clear effect

b) MD - tremors, twitches/flutterers, decreased motor activity, labored/rapid breathing, rough coat (M only), wet fur (M only), licking/chomping/chewing/gnawing on cage.

c) HD - above + flattened/hunched posture, ataxia, "unusual gait", depressed or lost righting reflex, salivation, piloerection, reduced feces, diarrhea/loose stool (M only), ptosis (F only).

d) No drug effect on incidence of palpable masses

2) Mortality

Slight decrease in HD F.

Numbers (%) alive at termination as follows:

	CONTROL 1	CONTROL 2	LD	MD	HD
M	15 (21%)	16 (23%)	19 (27%)	17 (24%)	15 (21%)
F	17 (24%)	12 (17%)	14 (20%)	13 (19%)	24 (34%)

Sponsor's plots of survival curves are attached.

In an 8 week gavage range finding study, deaths were seen at 10 mg/kg.

3) Bodyweight gain

Decreased at HD (mean near end of study ~ 10% below control) of both sexes and in MD F (~5-10%). See attached figures.

4) Food consumption

Slight decreases in groups with decreased bodyweight gain, above.

5) Organ weight

Not performed

6) Gross pathology

No drug effects

7) Histopathology

(Routine exam done in all animals).

There were no clear drug effects.

The incidence of mammary gland adenocarcinomas was, according to the sponsor's analysis, statistically significantly greater in HD F compared to the combined control group but not to the individual control groups. (Incidence in control group 1, control group 2, LD, MD, and HD was 0/70, 1/70, 1/71, 0/70, and 4/70, resp.) This result will be discussed in the Evaluation section of this review. There were no drug-related effects on the incidence of other neoplasms.

8) Toxicokinetics

Sampling was done from 5/sex/group, "approximately" 30 min. post-dose, weeks 23, 52, and 78. Blood was analyzed for parent compound and the metabolite ZNS 114-666. Limit of quantitation was . Sample tubes contained physostigmine to inhibit hydrolysis of parent compound.

Results (mean values) shown in attached table. For parent drug, most samples from LD and MD (and HD M, week 23 only) were below the limit of quantitation. Levels

of metabolite were considerably greater, and were generally proportional or somewhat less than proportional to dose. Blood levels of parent drug and metabolite were relatively constant across sampling times. (The increase in parent compound noted for HD M may be spurious due to the fact that most individual values at all times were below or just above the limit of quantitation; those which were below were assigned a value of 0.)

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Figure 1A

KAPLAN-MEIER PRODUCT LIMIT ESTIMATE GRAPHS OF ALL GROUPS (MALE)

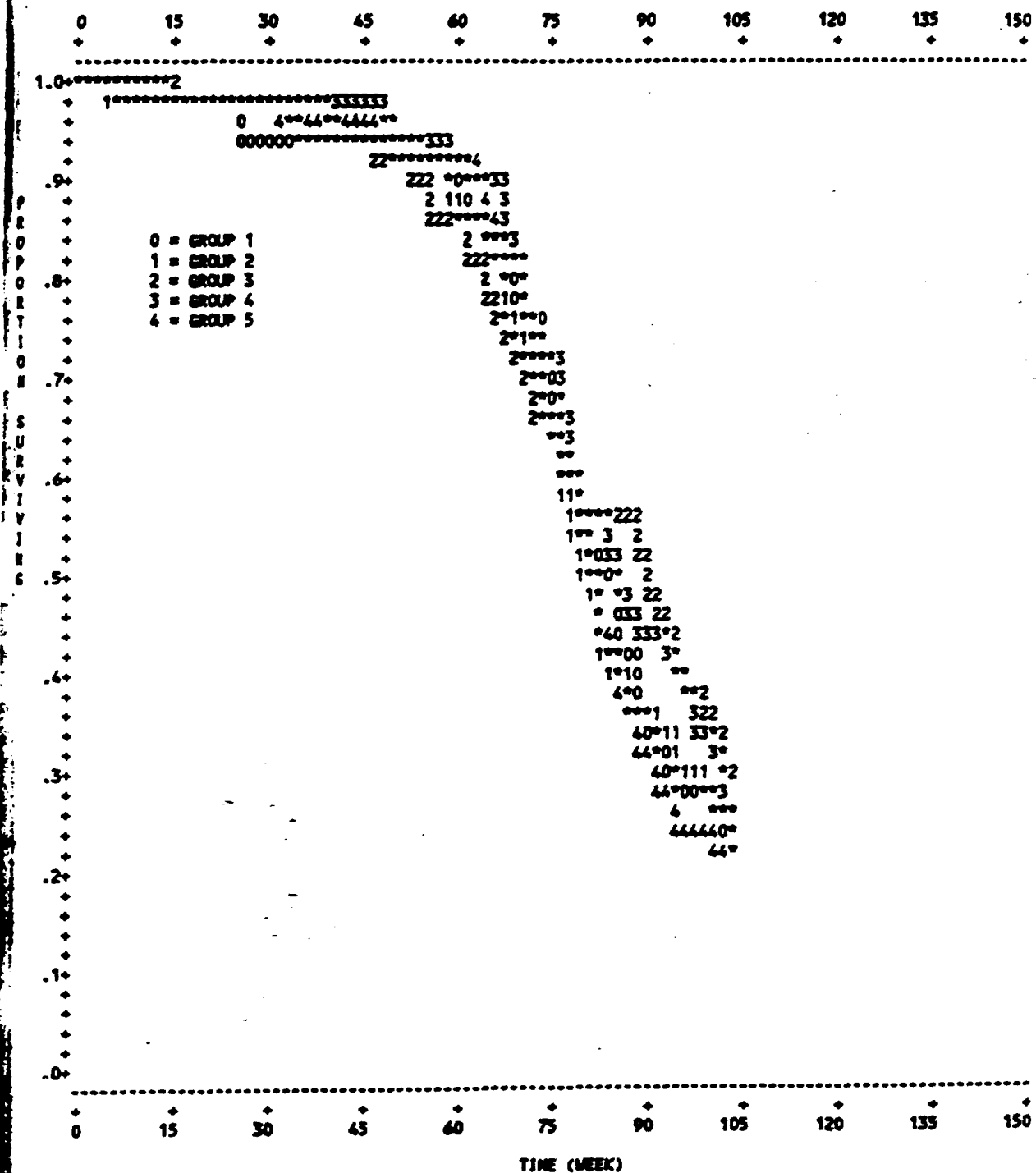


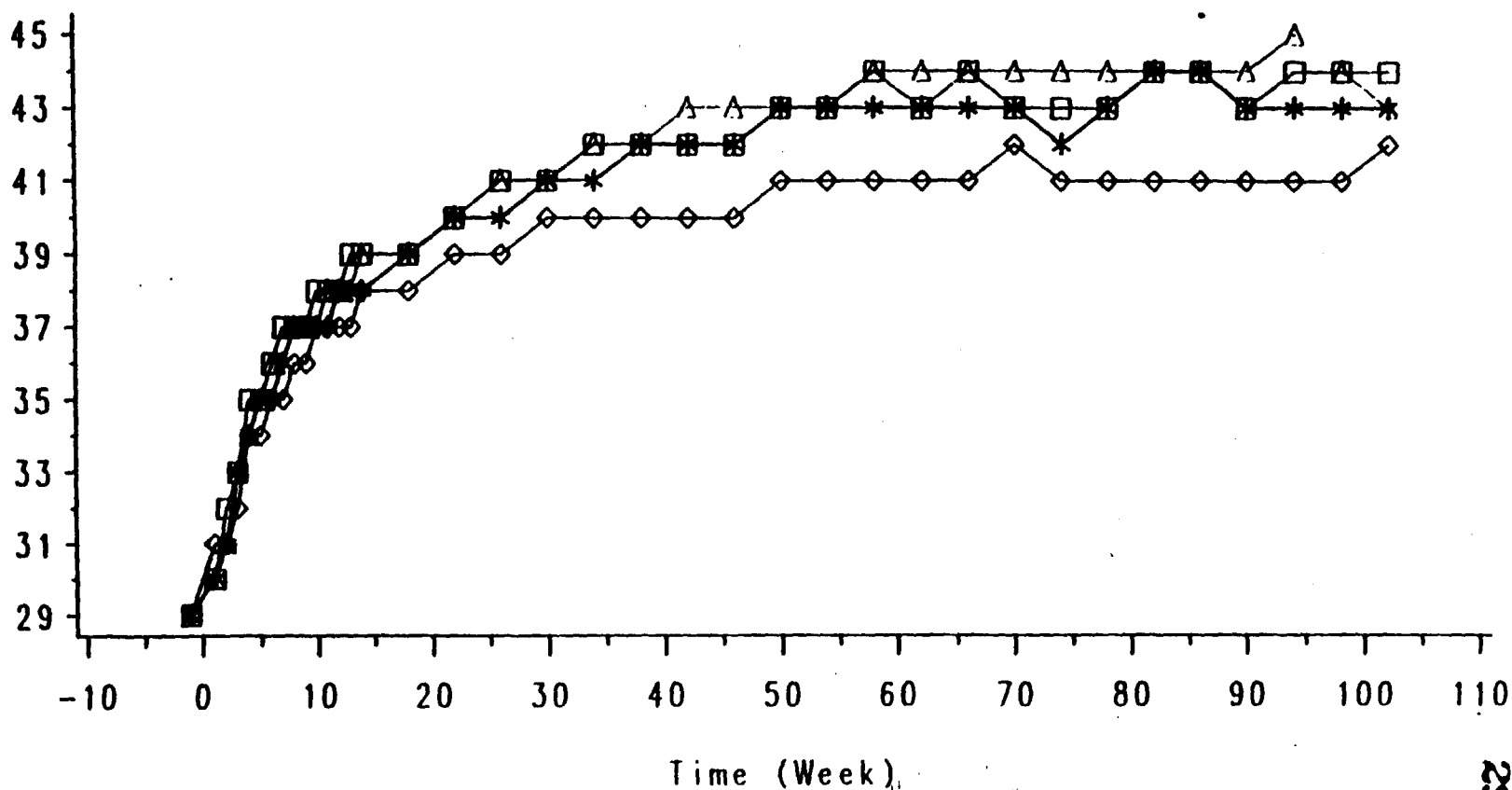
Figure AM-1

LIFETIME ORAL (GAVAGE) CARCINOGENICITY STUDY IN THE MOUSE
ON ENA 713
Sandoz Project T-2741

Body Weight Means

SEX=Male

Mean (GM)



GROUP

△ △ △ CONTROL I + II
* * * MID DOSE

□ □ □
◇ ◇ ◇

LOW DOSE
HIGH DOSE

Page 13 C

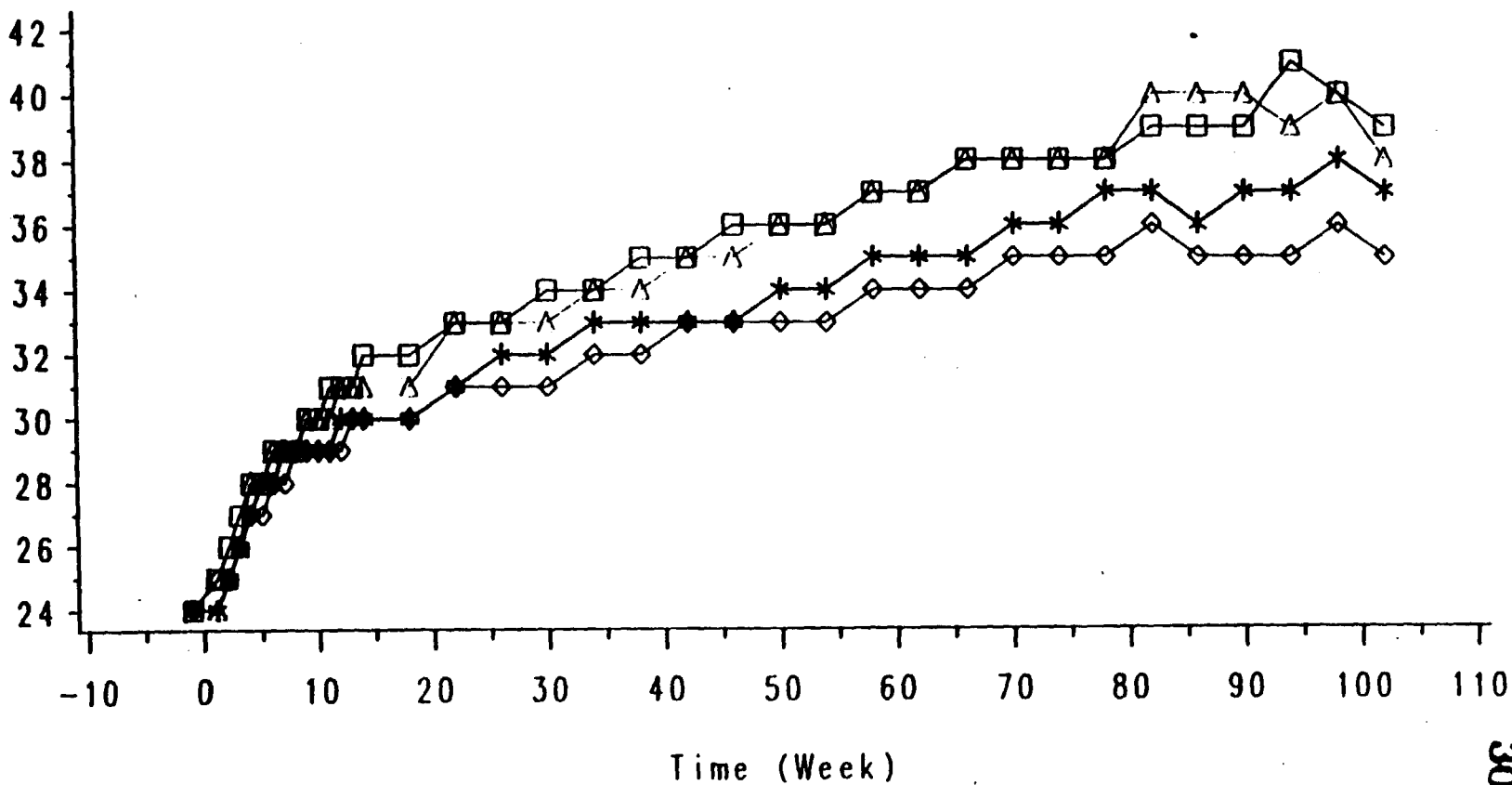
Figure AM-1

LIFETIME ORAL (GAVAGE) CARCINOGENICITY STUDY IN THE MOUSE
ON ENA 713
Sandoz Project T-2741

SEX=Female

Body Weight Means

Mean (GM)



GROUP

△ △ △ CONTROL I + II
* * * MID DOSE

□ □ □ LOW DOSE
◇ ◇ ◇ HIGH DOSE

LOW DOSE
HIGH DOSE

TABLE AM-4

**LIFETIME ORAL (GAVAGE) CARCINOGENICITY
STUDY IN THE MOUSE**

SANDOZ STUDY T-2741

Summary of Toxicokinetic Data

Mean Blood Concentration of SDZ ENA 713 (ng/mL)				
Dose (mg-base/kg/day)	Sex	Week 23	Week 52	Week 78
0.40	M	0	0	0
	F	0	0	0
1.0	M	0	0	0
	F	0	0	0
2.5	M	0.54	2.92	4.60
	F	3.25	4.34	2.08

Mean Blood Concentration of ZNS 114-666 (ng/mL)				
Dose (mg-base/kg/day)	Sex	Week 23	Week 52	Week 78
0.40	M	30.85	31.30	25.24
	F	40.89	52.53	41.36
1.0	M	38.75	80.60	37.84
	F	55.49	66.34	79.03
2.5	M	146.80	143.96	171.16
	F	263.64	249.02	168.24

Limit of quantitation =

q: U-2741, am-4

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ONE YEAR P.O. TOXICITY IN BEAGLE DOGS:

A) Dosage

4/sex at 0, 0.3, 0.6, and 2.5 → 2.1 mg/kg/day, in capsules.

(Doses expressed as hydrogen tartrate salt. Doses as free base = 0.2, 0.4, and 1.5 → 1.3 mg/kg, resp.)

Dosage change at HD occurred on day 4.

Drug lot # = 90904

B) Results

1) Observed signs

a) The following were seen roughly in decreasing frequency/incidence: tremors, lacrimation, injected sclera, salivation, (in M only) erythema, licking, decreased locomotor activity, increased locomotor activity, diarrhea, emesis/retching/gagging, increased respiration/labored breathing/coughing/rales/wheezing, ataxia, soiled/wet fur, unusual stance, bloody stool, injected mucus membranes.

In general, these signs were dose-related (an exception being increased locomotor activity, which was less prominent at HD, being replaced by decreased activity). Several signs (mainly those near the beginning of the above list) extended down to LD. According to the text, signs usually occurred from 0.5-2 hours post-dose with complete recovery by 4-6 hours, and the severity of signs "usually" diminished over the course of the study.

b) According to the text, clonic/tonic convulsions were seen in a HD F on 2 separate occasions (weeks 37 and 46, approx. 4 and 22 hours post-dose). One MD had "a single, brief episode of possible clonic/tonic seizures (approximately 1 minute in duration and approximately 4 hours post-dose) in week 39." Several HD animals were said to have "sporadic instances of unusual stance-rigid posture and forelimbs extended."

c) Periodic physical exams showed "poor reflexes (patellar, front placing, rear extensor, righting)" at HD, increased body temperature at MD and HD

(with a trend in LD F) (D-R, means generally 1-2° F above control), and no drug effect on heart rate. There were in general no statistically significant effects on respiratory rate, although mean values showed a trend toward higher values at MD and HD. (Individual values not given). (It was not stated at what time post-dosing any of the above measurements were made).

2) Mortality

One HD M died on day 2, and one HD M was sacrificed moribund on day 5, and one HD F died week 35. It was stated that the observed signs in the 2 dogs which died week 1 were of greater severity relative to the other HD dogs. For the dog which died on day 2, it was stated that "the probable cause of death was an intussusception resulting from intestinal hypermobility [sic]," considered to be an exaggerated pharmacological effect of the drug. It was stated that for the dog sacrificed on day 5, "a clear cause of death was not determined." For the dog which died week 35, it was stated that "the terminal event may have been cardiac hemorrhage, although this lesion could result of [sic] non-specific agonal hemorrhage." The 2 dogs which died during week 1 were replaced.

3) Bodyweight

Slight transient decrease in bodyweight at HD during first 1-2 weeks, followed by gain similar to that in controls.

4) Food Consumption

Decrease at HD, first week only.

5) Ophthalmoscopic exam

(Performed pre-study and weeks 3, 8, 12, 19, 26, and 52)

No drug effect (aside from lacrimation and injected sclera at all doses, as noted under "observed signs," above).

6) EKG

(Seven lead, performed pre-study and approx. 2 hours post-dosing in weeks 3, 7, 11, 18, 25, 37, and 51).

No drug effects on EKG or heart rate.

7) Hematology

(Done pre-study and weeks 4, 8, 12, 19, 26, 38, and 52).

At week 52, RBC slightly decreased in HD M, and Hb and Hct slightly decreased in MD and HD M. Very slight, non-statistically significant decreases in the above also seen at earlier times in these groups.

Other parameters measured/calculated: MCV, MCH, MCHC, reticulocytes, nucleated RBC, ESR, platelets, clotting time, PT, WBC, differential.

8) Blood chemistry

(Done at same times as hematology, above).

No clear drug effects, aside from decreases in "plasma cholinesterase." Mean values shown in attached table. Effects were mainly seen at HD, where means were ~ 1/2 control; this decrease was not progressive over time. The statistically significant decrease in LD and MD M were not much greater than those seen pre-study. There were no significant drug-related decreases in "RBC cholinesterase;" values in male drug groups were lower than controls both pre- and during treatment. (See attached table). (Note that the time of measurement of plasma or RBC "cholinesterase" relative to dosing was not stated, nor was methodology given. These factors could affect measured enzyme activity since the inhibition is reversible).

Other parameters measured: ALT, AST, AP, SDH, total bilirubin, total protein, glucose, cholesterol, triglycerides, urea nitrogen, electrolytes, calcium, magnesium.

9) Urinalysis

(Done at same times as hematology, above).

No drug effect

10) Organ weights

No drug effects.

11) Gross pathology

At the terminal sacrifice, 2 of the 3 HD F had the following findings on the serosal surface of the large intestine, "approximately 15 cm from the anus": "Flattened circular, firm, solitary, blood tinged nodules, ranging from 2 to 10 mm in diameter. The nodules were located approximately equidistant between the mesenteric side and the most antimesenteric region of the intestine. No macroscopic mucosal lesions were noted in the region of the serosal nodules."

In the HD F which died week 35, hemorrhages were seen on large intestine serosal surface, stomach muscularis, and heart. It was stated that "the terminal event may have been cardiac hemorrhage, although this lesion could result of [sic] non-specific agonal hemorrhage."

In the 2 HD M which died or were prematurely sacrificed during week 1, one had intussusception in small intestine (considered to be the cause of death), small and large intestinal mucosal congestion, and stomach glandular congestion. The other had small and large intestinal serosal hemorrhage.

12) Microscopic pathology

(Routine exam done in all animals).

At the terminal sacrifice, large intestinal serosal lesions consisting of "hemorrhage and fibrovascular proliferation (granulation tissue)" were seen in the 2 HD F with gross changes (see above) and in 1 HD M. In the F, lesions were described as "consisting of slightly elevated subserosal nodules containing hemorrhage, edema and a proliferation of both capillaries and fibroblasts in an organized 'granulation tissue' pattern." It was stated that "no lesions were noted in the muscular or mucosal regions in these sections of colon and there was no evidence of fibrin exudation or adhesion formation on the serosal surface." In the M, it was stated that "the lesion was primarily a deposition of hemosiderin and a cluster of mature capillaries within a subserosa neurovascular complex."

In the HD F which died week 35, the serosal surface of the large intestine had an area of hemorrhage and granulation tissue said to be similar to the lesions described above. Hemorrhage was also seen in heart, thymus and lymph node, as was lymphoid depletion in spleen and lymph node; these may be agonal events.

In the 2 HD M which died or were prematurely sacrificed during week 1, large intestinal serosal lesions similar to the above were not reported; findings in the animal with intussusception included G.I. congestion and small intestine mucosal and muscularis hemorrhage; findings in the other animal included small intestinal muscularis degeneration. One or both of these animals also had lymphoid depletion and lymph node hemorrhage.

There were no other clearly drug-related effects aside from the G.I. lesions noted above.

13) Toxicokinetics

Blood samples were collected from all dogs at 0.5, 1, 2, 4, 6, 8, and 24 hr. post-dose on day 1, week 24, and week 50, and at 1 hr. post-dose in weeks 5, 10, 17, and 36. Blood was analyzed for parent compound and metabolite ZNS 114-666. Limit of quantitation was

The sponsor's summary graphs of the day 1 and week 24 and 50 data are attached. Levels generally increased in proportion to or somewhat greater than proportional to dose. (Note that on day 1, MD and HD were assayed in a separate lab). Levels of the metabolite were greater than those of parent compound (AUC ~ 3-8x). Levels did not clearly change over time. (Again, note that MD and HD were assayed in a separate lab on day 1). (Lack of change over time also shown in the 1 hour assays at week 5, 10, 17, and 36; data not shown in attached graphs). Results were not summarized by gender.

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TABLE AM-4

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ONE YEAR ORAL TOXICITY STUDY
IN THE DOG ON BNA 713
SANDOZ PROJECT T-2723

CLINICAL CHEMISTRY PARAMETERS (MEAN VALUES)

PARAMETER	DRUG WEEK	GROUP I CONTROL 0 MG/KG/DAY		GROUP II LOW DOSE 0.3 MG/KG/DAY		GROUP III MID DOSE 0.6 MG/KG/DAY		GROUP IV HIGH DOSE 2.1 MG/KG/DAY	
		M	F	M	F	M	F	M	F
RBC CHOLINESTERASE IU/L	-3	1929.	1365.	1245.	1410.	1545.	1395.	1133.	1220.
	-1	1425.	1013.	990.	975.	1110.	945.	983.	675.
	4	1748.	1463.	1170.	1418.	1365.	1260.	1110.	1163.
	8	1890.	1365.	1275.	1453.	1530.	1313.	1073.	1200.
	12	1883.	1448.	1463.	1493.	1665.	1353.	1328.	1313.
	19	1920.	1665.	1778.	1770.	2025.	1815.	1470.	1493.
	26	1635.	1133.	1290.	1523.	1583.	1305.	1148.	1283.
	38	2070.	1493.	1553.	1673.	1815.	1485.	1305.	1310.
	52	1875.	1320.	1433.	1515.	1585.	1343.	1133.	1130.
CALCIUM mg/dl	1	12.2	12.0	11.6	11.9	11.4	11.3	11.3	12.1
	2	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	3	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	4	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	5	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	6	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	7	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	9	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	10	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8

*not
thru*

* STATISTICALLY ($P < 0.05$) DIFFERENT FROM CONTROL BY ANOVA FOLLOWED BY DUNCAN'S
NEW MULTIPLE RANGE TEST.

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ONE YEAR ORAL TOXICITY STUDY
IN THE DOG ON RWA 713
SANDOZ PROJECT T-2723

CONFIDENTIAL-TRADE SECRET

CLINICAL CHEMISTRY PARAMETERS (MEAN VALUES)

PARAMETER	DRUG WEEK	GROUP I CONTROL 0 MG/KG/DAY		GROUP II LOW DOSE 0.3 MG/KG/DAY		GROUP III MID DOSE 0.6 MG/KG/DAY		GROUP IV HIGH DOSE 2.1 MG/KG/DAY	
		M	F	M	F	M	F	M	F
TRIOSTENINE NG/ML	3		45.		42.	31.	42.		35.
	1		45.		42.	31.	42.		35.
	4		45.		42.	31.	42.		35.
	8		45.		42.	31.	42.		35.
	12		45.		42.	31.	42.		35.
	16		45.		42.	31.	42.		35.
	20		45.		42.	31.	42.		35.
	24		45.		42.	31.	42.		35.
	28		45.		42.	31.	42.		35.
	32		45.		42.	31.	42.		35.
	36		45.		42.	31.	42.		35.
	40		45.		42.	31.	42.		35.
PLASMA CHOLINE, IU/L	3	1937.	1593.	1399.	1629.	1400.	1651.	1673.	1514.
	1	1936.	1652.	1482.	1575.	1382.	1675.	1762.	1557.
	4	2388.	1720.	1274.	1556.	1128.	1349.	1047.	964.
	8	1984.	1801.	1258.	1972.	1137.	1357.	1029.	990.
	12	2073.	1560.	1254.	1554.	1147.	1573.	986.	1029.
	16	1920.	1851.	1404.	1448.	1102.	1371.	976.	861.
	20	2061.	1666.	1262.	1472.	1134.	1479.	1013.	898.
	24	2004.	1906.	1210.	1569.	1050.	1363.	999.	964.
	28	2062.	1656.	1213.	1399.	1066.	1504.	1009.	951.
	32								
	36								
	40								

~~_____~~

Check

(~~part~~ shows lot
of ~~materials~~)

~~not needed~~
~~no party~~

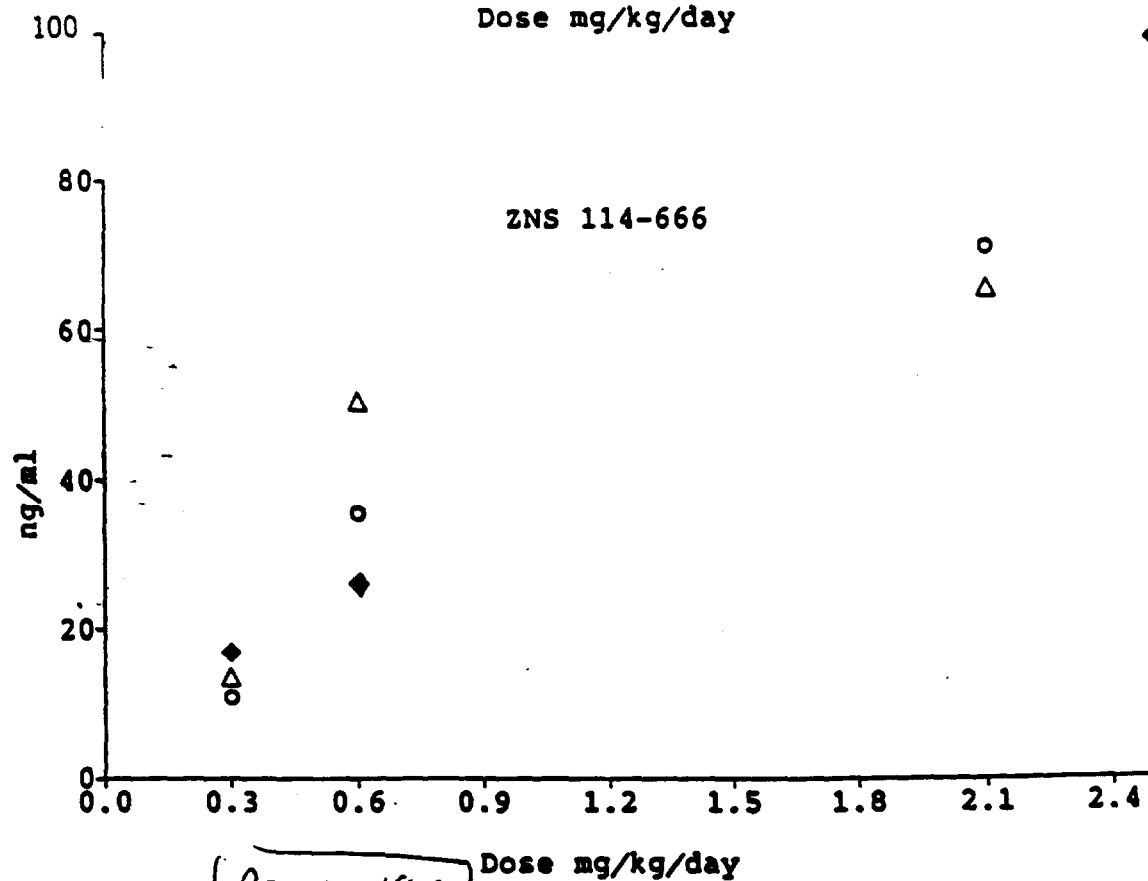
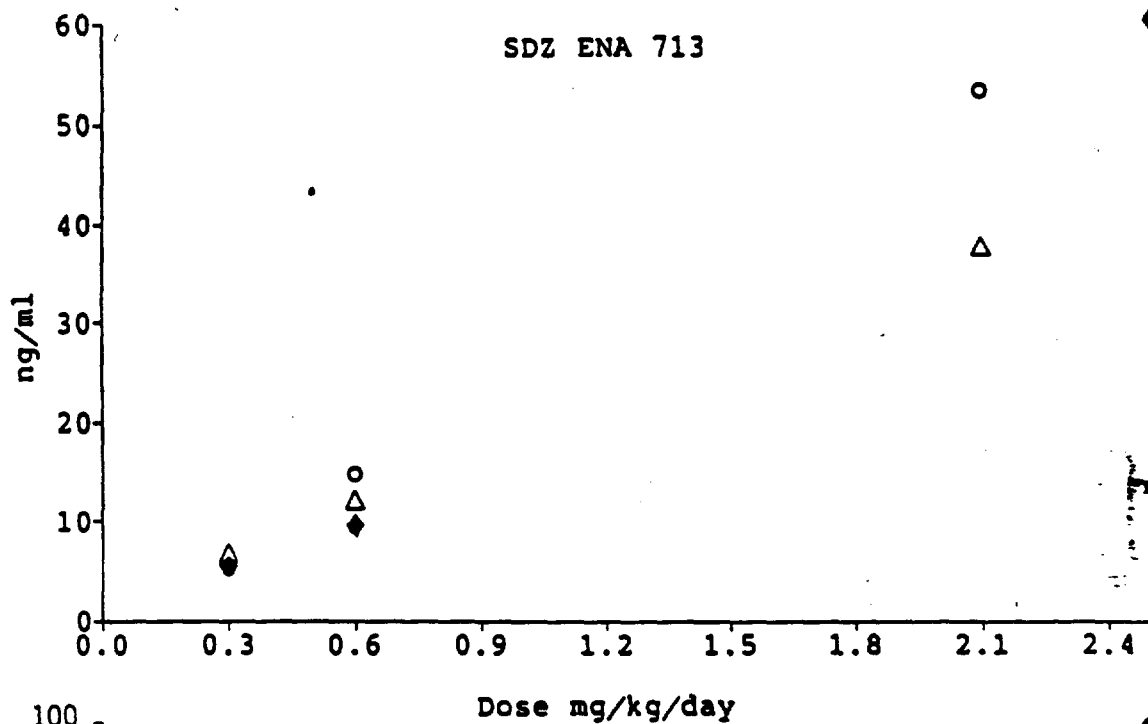
* STATISTICALLY ($P < 0.05$) DIFFERENT FROM CONTROL BY ANOVA FOLLOWED BY DUNCAN'S NEW MULTIPLE RANGE TEST.

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Figure 1

ONE YEAR ORAL TOXICITY STUDY IN THE DOG (T-2723)

C MAX



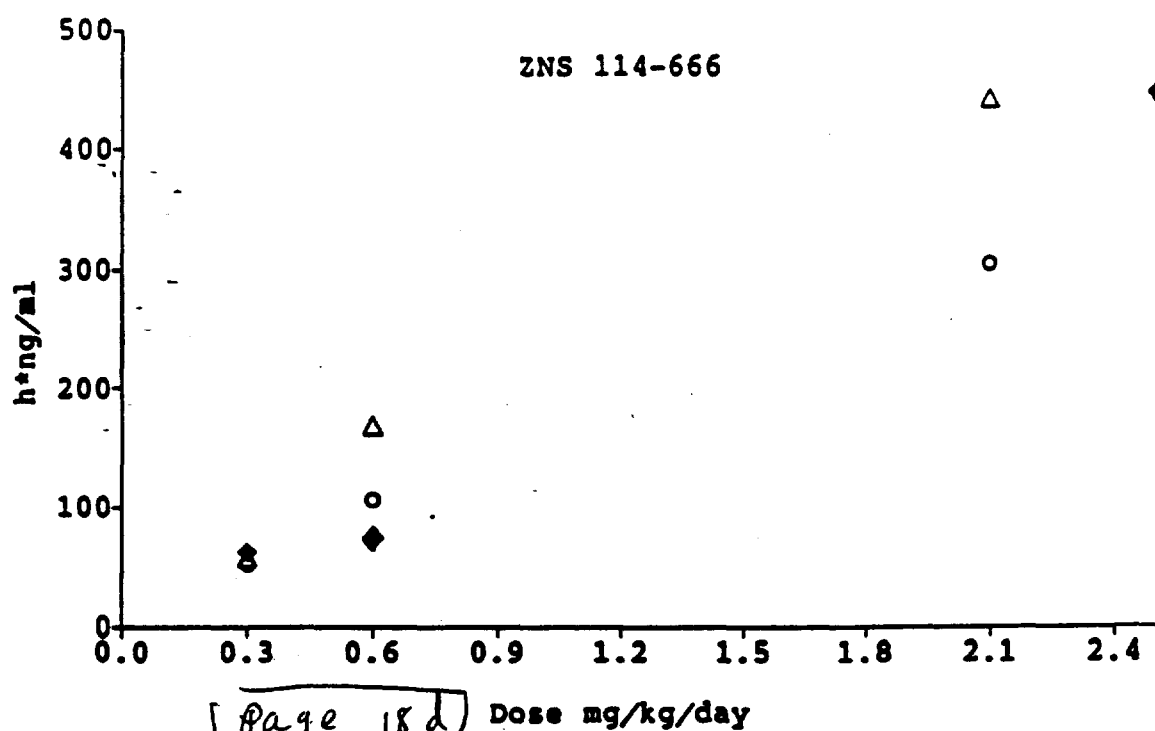
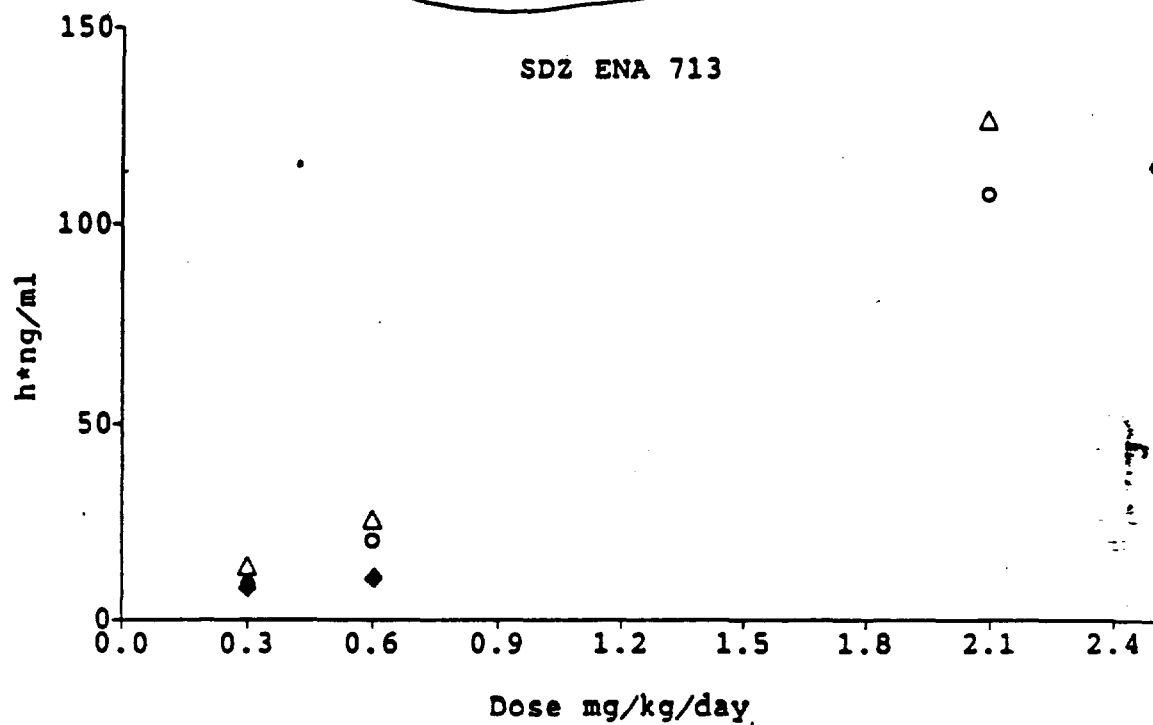
page 18c

◆ W 01
○ W 24
△ W 50

Figure 2

ONE YEAR ORAL TOXICITY STUDY IN THE DOG (T-2723)

A.U.C.(0-24h)



SEGMENT I REPRODUCTION IN RATS:

A) Dosage

25/sex at 0, 0.2, 0.9, and 1.8 mg/kg/day, by gavage.

(Doses given as hydrogen tartrate salt. Doses as free base = 0.12, 0.55, and 1.1 mg/kg).

M were dosed from 10 weeks pre-mating through necropsy at study week 21. F were dosed from 2 weeks pre-mating through either day 20 of gestation (in 12 F/group, which underwent C section) or through day 21 PP (in remaining F, which delivered naturally). (Evidence of sperm in vaginal smears or presence of plug = day 0 of gestation).

Among F sacrificed on day 20, approx. 1/3-1/2 fetuses in each litter underwent visceral exam (cross-sectioning and dissection), and the remaining fetuses underwent skeletal exam (Alizarin Red S staining). Among F allowed to deliver naturally, pups were evaluated for various developmental milestones, cognitive ability, and fertility as shown on attached page.

Strain: _____

Drug lot #: 88902

B) Results

1) Observed signs in F₀ animals

Only narrative description was given. The following were said to have occurred at HD and, at a lower incidence, at MD: ataxia, whole body twitches/flutterers, tremors, decreased locomotor activity, flattened body position, salivation, licking, tail biting, chomping/chewing, excessive grooming, piloerection, gnawing on digits. The following were seen in M only: tactile hypersensitivity, lacrimation, loose stool, wet fur, red substance around nares, increased (in addition to decreased) locomotor activity, and rales.

2) Mortality of F₀

No drug effects.

(Three controls, 1 LD, and 1 HD died; all were males).

3) F₀ bodyweight

a) Males: No clear drug effect. Mean weights at HD were occasionally

statistically significantly less than controls but a similar (non-significant) decrease was seen pre-treatment (93% of control).

- b) Females: According to the text, weight gain was decreased at MD and HD during the pre-mating period. Mean weights at the end of this period were ~ 90% of control. These differences were maintained through the gestation period but were lessened during the postpartum period (i.e., MD and HD tended to gain more weight than controls during the latter). Note that the decreased weight gains were generally similar in magnitude between MD and HD; also note that it is not clear if the initial values in the table, which were 2-3% below control at MD and HD, represent pre-treatment weights or weights during the first week of treatment; if the former, it indicates that the drug effect was less than that indicated above; if the latter, then nothing at all can be concluded about drug effects since a similar difference might have existed pre-treatment.

4) **F₀ food consumption**

- a) Males: Text states consumption was decreased at MD and HD; however, at MD pre-treatment consumption was lower by a similar amount. At HD, consumption was ~ 90% of control, vs 96% of control pre-treatment.
- b) Females: Consumption was decreased at MD and HD during the pre-mating period (83 and 77% of control, resp.; and to a lesser degree during gestation (94 and 87%, resp.); however, as with body weights, it is unclear if or to what degree there were any pre-treatment differences. (The initial values in the table show consumption to be 86 and 79% of controls at MD and HD, resp.). There were no clear drug effects on food consumption during the postpartum period.

5) **F₀ fertility and other reproductive parameters:**

Some of the sponsor's summary tables are attached. There were no drug effects on estrus cycles, fertility, pre- and post- implantation loss, etc. (Among F sacrificed on day 20, there were non-statistically significant decreases in CL, implantation sites, and litter size at all doses, generally not D-R; however, a trend in the opposite direction was seen among F allowed to deliver naturally).

Exams of fetuses from C-sectioned animals showed no drug - related malformations or variations. (Tables not included in present review).

Among F allowed to deliver naturally, there were no drug effects on pup survival

through day 21 PP. Pup weights through day 21 PP were slightly decreased at all doses; this was not statistically significant but was dose - related and considered by the sponsor to suggest a drug - related trend. (Mean weight at HD on day 21 PP was 10% below control. See table 20). (Among pups selected for further evaluation after day 21 PP, this slight trend continued among M through the last measurement time [day 91 PP], but disappeared in F).

6) **F₁ developmental and fertility data**

Aside from the slight, equivocal decreases in bodyweights noted above, there were no effects on F₁ development, behavioral performance, or reproductive performance.

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Segment I rat

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TEST OR MEASUREMENT

AGE (DAYS POSTPARTUM)

Pre-Weaning Evaluation (All Pups)

Viability, observations	0-21
Sex ratio, individual weights	0, 1, 4, 7, 14, 21
Pinna unfolding	2 to criterion
Surface righting reflex	3 to criterion
Negative geotaxis	5 to criterion
Acoustic (Auditory) startle	10 to criterion
Eye opening	13 to criterion

Post-Weaning Evaluation (Two Pups Per Litter)

Observations	Daily from day 21 pp until necropsy
Pupil constriction	21 to criterion
Testes descent	21 to criterion
Vaginal opening	30 to criterion
Learning ability	27
Memory capacity	28
Open field	42
Body weight	21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91
Fertility	Start mating day 91 pp

TABLE 4

INVESTIGATION OF THE EFFECTS OF ENA 713 ON THE FERTILITY
AND REPRODUCTION PERFORMANCE IN THE RAT - SEGMENT 1
SANDOZ PROJECT T-2676P

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SUMMARY OF ESTROUS CYCLE STAGES

DOSAGE		0 MG/KG /DAY	0.2 MG/ KG/DAY	0.9 MG/ KG/DAY	1.8 MG/ KG/DAY
FEMALES EXAMINED	N	25	25	25	25
MEAN # OF DAYS OF STAGE 1 OBSERVED		3.6	3.4	4.2	3.8
S.D.		1.3	1.6	1.3	1.3
MEAN # OF DAYS OF STAGE 2 OBSERVED		3.9	3.6	3.5	3.3
S.D.		1.2	1.1	1.5	1.2
MEAN # OF DAYS OF STAGE 3 OBSERVED		3.0	3.0	3.0	3.3
S.D.		1.4	1.7	1.1	1.0
MEAN # OF DAYS OF STAGE 4 OBSERVED		3.4	3.3	3.2	3.6
S.D.		1.1	1.2	1.2	1.6
FEMALES WITH ALL STAGES	N	24	25	24	25
	n	96	100	96	100

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

STAGE 1 = PROESTRUS, STAGE 2 = ESTRUS, STAGE 3 = METESTRUS, STAGE 4 = DIESTRUS

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TABLE 13

INVESTIGATION OF THE EFFECTS OF KHA 713 ON THE FERTILITY
AND REPRODUCTION PERFORMANCE IN THE RAT - SEGMENT I
SANDOZ PROJECT T-2676P

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SUMMARY OF OVERALL MATERNAL FERTILITY AND FETAL DATA AT TIME OF CESAREAN SECTION - P₀ MATING

DOSAGE		0 MG/KG /DAY	0.2 MG/ KG/DAY	0.9 MG/ KG/DAY	1.8 MG/ KG/DAY
Females Mated	N	24	25	25	25
Pregnant	N	20	21	20	23
Aborted	N	0	0	0	0
Premature Births	N	0	0	0	0
Dams with Viable Fetuses	N	10	11	10	11
Dams with all Resorptions	N	0	0	0	0
Female Mortality	N	0	0	0	0
Pregnant at C-section	N	10	11	10	11
Corpora Lutea	N	201	199	102	100
	MEAN	20.1	19.1	10.2	10.0
	S.D.	3.1	4.7	2.9	3.7
Implantation Sites	N	175	151	134	154
	MEAN	17.5	15.1	13.4	15.4
	S.D.	1.6	6.4	4.7	5.5
Preimplantation Loss	%	12.9	24.1	26.4	14.4
Postimplantation Loss	%	0.6	0.6	3.0	1.9
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	15	13	4	6
	%	0.6	0.6	3.0	1.9
	MEAN	1.5	1.2	0.4	0.5
	S.D.	1.4	1.0	0.7	0.9
Early Resorptions	N	15	13	4	6
	%	0.6	0.6	3.0	1.9
	MEAN	1.5	1.2	0.4	0.5
	S.D.	1.4	1.0	0.7	0.9
Late Resorptions	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0

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

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TABLE 13

INVESTIGATION OF THE EFFECTS OF ENA 713 ON THE FERTILITY
AND REPRODUCTION PERFORMANCE IN THE RAT - SEGMENT I
SANDOZ PROJECT T-2676P

CONFIDENTIAL-TRADE SECRET

SUMMARY OF OVERALL MATERNAL FERTILITY AND FETAL DATA AT TIME OF CESAREAN SECTION - P₀ MATING

DOSAGE		0 MG/KG /DAY	0.2 MG/ KG/DAY	0.9 MG/ KG/DAY	1.8 MG/ KG/DAY
Viable Fetuses	N	160	138	130	148
	%	91	91	97	96
	MEAN	16.0	12.5	13.0	13.5
	S.D.	2.2	5.8	5.0	5.4
Viable Male Fetuses	N	74	77	61	64
	%	46	56	47	43
Live Fetal Body Weight (g)	MEAN	3.9	4.1	4.0	4.0
	S.D.	0.4	0.4	0.3	0.3
Male Fetuses	MEAN	4.0	4.2	4.2	4.1
	S.D.	0.5	0.4	0.3	0.3
Female Fetuses	MEAN	3.8	3.9	3.9	3.9
	S.D.	0.3	0.3	0.3	0.2

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

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TABLE 20

INVESTIGATION OF THE EFFECTS OF ERA 713 ON THE FERTILITY
AND REPRODUCTION PERFORMANCE IN THE RAT - (SEGMENT 1)
SANDOZ PROJECT T-2676V

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NATURAL DELIVERY AND LITTER DATA -- SUMMARY

	DOSE	0 MG/KG /DAY	0.2 MG/ KG/DAY	0.9 MG/ KG/DAY	1.8 MG/ KG/DAY
Females Mated	N	13	13	13	13
Females Pregnant	N	10	10	10	12
	%	77	77	77	92
Females Surviving Delivery	N	9	10	10	12
	%	90	100	100	100
Duration of Gestation	MEAN	22.1	21.9	21.9	21.9
	S.D.	0.3	0.3	0.3	0.3
with Stillborn Pups	N	1	0	1	2
	%	11	0.0	10	17
with all Stillborn/Uncertain	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
with one or more Liveborn	N	9	10	10	12
	%	100	100	100	100
with Entire Liveborn Litter Dying and/or Missing, Cannibalized, Culled					
days 0-4	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
days 5-21	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
days 0-21	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

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

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TABLE 20

INVESTIGATION OF THE EFFECTS OF ENA 713 ON THE FERTILITY
AND REPRODUCTION PERFORMANCE IN THE RAT - SEGMENT I
SANDOZ PROJECT T-2676P

CONFIDENTIAL-TRADE SECRET

NATURAL DELIVERY AND LITTER DATA -- SUMMARY

DOSAGE		0 MG/KG /DAY	0.2 MG/ KG/DAY	0.9 MG/ KG/DAY	1.0 MG/ KG/DAY
Litters Delivered (total)	N	9	10	10	12
Pups Delivered (total)	N	113	136	140	166
	MEAN	12.6	13.6	14.0	13.8
	S.D.	3.7	3.9	3.6	2.6
Liveborn	N	112	136	130	163
Stillborn	N	1	0	2	2
	%	0.9	0.0	1.4	1.2
Uncertain	N	0	0	0	1
Culled day 1	N	48	37	39	61
Culled (total)	N	48	37	39	61
Cannibalized	N	0	0	0	0
Missing	N	2	1	0	4
Liveborn, not culled prior to day 21	N	64	79	79	102
Pups Dying, Missing, and/or Cannibalized day 0	N	0	0	1	1
	%	0.0	0.0	0.7	0.6
days 1-4	N	1	1	1	3
	%	0.9	0.7	0.7	3.1
days 5-7	N	2	0	0	0
	%	1.6	0.0	0.0	0.0
days 8-14	N	0	1	2	0
	%	0.0	0.7	1.4	0.0
days 15-21	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pups Surviving 21 days	N	61	77	75	96
	%	93	97	95	94
Implantation Sites per Litter	N	122	145	153	176
	MEAN	13.6	14.5	15.3	14.7
	S.D.	6.0	3.9	3.4	3.0

SIGNIFICANTLY DIFFERENT FROM CONTROL; * - $P < 0.05$; ** - $P < 0.01$.

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TABLE 20

CONFIDENTIAL-TRADE SECRET

INVESTIGATION OF THE EFFECTS OF ERA 713 ON THE FERTILITY
AND REPRODUCTION PERFORMANCE IN THE RAT - (SEGMENT I)
SANDOZ PROJECT T-2676P

NATURAL DELIVERY AND LITTER DATA -- SUMMARY

DOSAGE		0 MG/KG /DAY	0.2 MG/ KG/DAY	0.9 MG/ KG/DAY	1.6 MG/ KG/DAY
Live Pups/Litter					
day 0	MEAN	12.4	13.6	13.0	13.6
	S.D.	5.7	3.9	3.5	2.9
day 1 - Pre-culling	MEAN	12.3	13.5	13.6	13.1
	S.D.	5.5	3.0	3.5	3.0
day 1 - Post-culling	MEAN	7.0	7.0	7.7	8.0
	S.D.	2.1	0.6	0.9	0.0
day 4	MEAN	7.0	7.0	7.7	8.0
	S.D.	2.1	0.6	0.9	0.0
day 7	MEAN	6.8	7.8	7.7	8.0
	S.D.	2.1	0.6	0.9	0.0
day 14	MEAN	6.8	7.7	7.5	8.0
	S.D.	2.1	0.7	1.0	0.0
day 21	MEAN	6.8	7.7	7.5	8.0
	S.D.	2.1	0.7	1.0	0.0
Pup Weight/Litter (grams)					
day 0	MEAN	6.7	6.6	6.4	6.3
	S.D.	0.6	0.3	0.5	0.5
day 1	MEAN	7.5	7.2	7.2	7.1
	S.D.	0.6	0.6	0.6	0.5
day 4	MEAN	12.2	12.0	11.3	11.2
	S.D.	1.3	1.0	1.1	0.8
day 7	MEAN	16.3	17.7	17.1	16.5
	S.D.	1.9	1.5	1.9	1.7
day 14	MEAN	35.1	34.4	34.0	31.4
	S.D.	6.4	1.9	2.9	3.4
day 21	MEAN	50.3	56.0	56.0	52.6
	S.D.	10.4	2.6	4.7	5.5
Sex Ratio - Male Pups/Total Pups					
day 0	N	62	67	67	71
	%	55	49	49	44
day 21	N	32	36	36	44
	%	52	47	40	46

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

SEGMENT II REPRODUCTION IN RATS (1st of 2 STUDIES):

A) Dosage

25 F at 0, 0.4, 1.2, or 3.6 mg/kg/day, by gavage.

(Doses given as hydrogen tartrate salt. Doses as free base = 0.24, 0.72, and 2.2 mg/kg).

Dosing was days 6-15 of gestation. (Day of finding vaginal plug=day 0 of gestation).

Dams were sacrificed day 20 of gestation. Following external exam of fetuses, approx. 1/3-1/2 of fetuses per litter were examined visceraally (cross-sectioning and dissection); the remaining fetuses were examined skeletally. (Attached page shows numbers of pregnant dams, and numbers of fetuses examined, in each group).

Strain: _____

Drug lot #: 88902

B) Results

1) Observed signs in dams

Narrative summary only. Signs at HD were said to include ataxia, tactile/auditory hypersensitivity, whole body twitches, flutters and tremors, decreased locomotor activity, flattened body position, salivation, licking, biting cage, chomping/chewing, lacrimation, piloerection, loose stool, and reduced feces). It was stated that "a few" MD had "similar signs," and that no drug effects were seen at LD.

2) Dam mortality

None

3) Dam bodyweight

Decreased gain at MD and HD. At day 15 of gestation, mean weights were 96% and 90% of control at MD and HD, resp. Weight gain between days 15 and 20 was similar across groups. (It is noted that weight gain during days 0-6 of gestation, i.e. prior to treatment, was slightly less [but statistically significant] at HD than in controls, such that by the beginning of treatment [day 6] the mean weight at HD was 98% of control.

After subtracting gravid uterus weight from day 20 bodyweight, weights were still ~~below~~ control at MD and HD but only statistically significant in the latter.

4) **Dam food consumption**

Decreased at MD and HD. (Mean ~ 90% and 75% of control during treatment).

5) **Reproductive parameters**

No drug effects on post-implantation loss, resorptions, or live or dead fetuses. Fetal weight was equivocally decreased at HD (mean 94% of control) (See table 7, attached).

6) **Fetal exam**

The only drug - related effects were slight increases in the following skeletal variations at HD, considered to represent delayed skeletal development (secondary to maternal toxicity):

a) **Hyoid body not ossified**

Fetal and litter incidence at HD = 25% and 75%, resp.; corresponding values in control = 16 and 50%.

b) **5th sternebra not ossified**

Fetal and litter incidence at HD = 54 and 96%, resp.; corresponding values in control = 43 and 86%. (Incidence of not ossified 6th sternebra also slightly increased, but not statistically significant).

Rat Segment II

CONFIDENTIAL-TRADE SECRET

(1st of 2 studies)

IV. METHODS

A. EXPERIMENTAL DESIGN

The purpose of this investigation was to detect possible effects on the developing rat fetuses after oral administration of various dose levels of ENA 713 to dams during the period from Days 6 through 15 of pregnancy.

The following table summarizes groups, doses, female animal identification and numbers of animals (and fetuses) evaluated:

Group Dose (mg/kg/day) ^a	Control 0	Low 0.4	Mid 1.2	High 3.6
Triturated Dose (mg/kg/day) ^b	32.4	4	12	36
Animal #89- (female-odd numbers)	2585-2633	2635-2683	2685-2733	2735-2783
# Animals Mated	25	25	25	25
# Animals Pregnant	22	21	21	24
# Pregnant Animals Examined on Day 20	22	21	21	24
<u>Fetal Exam</u>				
Total # External Exam ^c	326	306	286	341
Total # Visceral Exam - Bouin's fixed	126	121	112	133
Total # Skeletal Exam - Alizarin Red-S	200	185	174	208
Total # fetuses not suitable for skeletal or visceral exam (e.g. late resorptions)	0	0	0	0

^aThese salt dose levels represent equivalent base dose levels (using salt factor 1.65) of 0.24, 0.72 and 2.2 mg/kg/day for the Low, Mid and High dose groups, respectively.

INVESTIGATION OF TERATOGENIC POTENTIAL OF 212-712
(ENA 713) IN THE RAT - SEGMENT II
SANDOZ PROJECT T-2618

TABLE 7

SUMMARY OF MATERNAL AND FETAL DATA AT CESAREAN SECTION

DOSEAGE		0 MG/KG /DAY	0.4 MG/ KG/DAY	1.2 MG/ KG/DAY	3.6 MG/ KG/DAY
Females Mated	N	25	25	25	25
Pregnant	N	22	21	21	24
Aborted	N	88	84	84	96
Premature Births	N	0	0	0	0
Dams with Viable Fetuses	N	0	0	0	0
Dams with all Resorptions	N	22	21	21	24
	N	0	0	0	0
Female Mortality	N	0	0	0	0
Pregnant at C-section	N	22	21	21	24
Corpora Lutea	N	416	363	362	443
	MEAN	18.9	17.3	17.2	18.5
	S.D.	3.7	2.6	2.2	3.7
Implantation Sites	N	355	325	310	359
	MEAN	16.1	15.5	14.8	15.0
	S.D.	2.1	2.7	2.2	3.3
Preimplantation Loss	%	14.7	10.5	14.4	19.0
Postimplantation Loss	%	8.2	6.2	7.7	5.0
Dead Fetuses	N	0	1	0	0
	%	0.0	0.3	0.0	0.0
Resorptions, total	N	29	19	24	18
	%	8.2	5.8	7.7	5.0
	MEAN	1.3	0.9	1.1	0.8
	S.D.	1.5	0.8	1.7	0.9
Early Resorptions	N	29	19	24	18
	%	8.2	5.8	7.7	5.0
	MEAN	1.3	0.9	1.1	0.8
	S.D.	1.5	0.8	1.7	0.9
Late Resorptions	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0

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

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INVESTIGATION OF TERATOGENIC POTENTIAL OF 212713
(BNA 713) IN THE RAT - SEGMENT II
SANDOZ PROJECT T-2618

(CONT'D) - TABLE 7

SUMMARY OF MATERNAL AND FETAL DATA AT CESAREAN SECTION

DOSAGE		0 MG/KG /DAY	0.4 MG/ KG/DAY	1.2 MG/ KG/DAY	3.6 MG/ KG/DAY
Viable Fetuses	N	326	305	286	341
	%	92	94	92	95
	MEAN	14.8	14.5	13.6	14.2
	S.D.	2.4	2.6	2.9	3.2
Viable Male Fetuses	N	169	146	143	158
	%	52	48	50	48
Live Fetal Body Weight (g)	MEAN	3.6	3.7	3.6	3.4
	S.D.	0.3	0.2	0.3	0.2
Male Fetuses	MEAN	3.7	3.8	3.7	3.6
	S.D.	0.3	0.2	0.3	0.3
Female Fetuses	MEAN	3.5	3.6	3.9	3.4
	S.D.	0.3	0.2	0.3	0.2

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

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SEGMENT II REPRODUCTION IN RATS (2nd of 2 STUDIES):

A) Dosage

38 F at 0, 0.4, 1.3, and 4.0 mg/kg/day, by gavage

(Doses expressed as hydrogen tartrate salt).

Dosing was days 7-17 of gestation (Day of finding evidence of copulation = day 0 of gestation).

Approx. 2/3 of pregnant dams sacrificed day 20 of gestation; all fetuses examined externally, approx. 1/3 viscera (), and approx. 2/3 skeletally (). Remaining F allowed to deliver naturally, with pup evaluation including various development milestones, and behavioral and reproductive performance. The actual numbers of pregnant F evaluated were as follows:

	<u>C-SECTION</u>	<u>NATURAL DELIVERY</u>
Control	25	13
LD	24	13
MD	24	12
HD	24	13

Strain: Crj: /

Drug batch#: 90904

B) Results

1) Observed signs in dams

a) LD - miosis

b) MD - miosis (+ salivation and tremors on a single day in a single animal, and tremors on a single day in another animal)

c) HD - miosis, tremors, salivation, decreased spontaneous motor activity, lacrimation, and, in 3 animals on single occasions, irregular respiration.

2) Dam mortality

None

3) Dam bodyweight

Decreased gain at MD and HD. Mean weights on day 18 of gestation ~ 94% and 92% of control, resp. Little or no differences from control by day 21 PP.

4) Dam food consumption

Decreased at MD and HD during treatment period. (Mean values ~ 85 and 75-85% of control, resp.) No consistent differences from control during lactation period.

5) Results in Fo dams sacrificed day 20 of gestation

a) Increased early resorptions at HD, but no effect on numbers of live fetuses. Placental weight also slightly decreased at HD. Fetal weight equivocally decreased at HD (mean 93% of control). (See table 4, attached) (It was stated that in a preliminary study at 1, 2, 4, and 8 mg/kg, the HD caused decreased weights of fetuses and placenta, as well as maternal weight loss).

b) Fetal Exam

Results shown in attached tables 5-1 thru 5-3 (which also show numbers of fetuses examined). It was concluded that there were no drug effects. (Table "5-2" shows an increased incidence of skeletal variations, primarily due to an increase in "lumbar rib", at HD. According to the text, the incidence of skeletal variations at HD, 9.5%, is within the historical control range of the lab [mean 6.9 %, range 1.4-16.9 %]). (Note that the bones showing decreased ossification in the first segment II study were not listed in the results). (Also note that it was stated that in the above-mentioned preliminary study, the HD [8mg/kg] caused retardation of fetal ossification, but no specifics were given).

6) Results in Fo dams allowed to deliver naturally

a) No drug effects on litter size, live or stillborn pups, or pup survival or weights through day 21 PP. (See sponsor's table 6 and 7, attached). There were also no group differences in weights measured through week 8 PP; however, note that among female pups chosen for mating at week 11 PP, weights were 5-15% below controls

in all groups, continuing through the gestation and lactation periods [greatest effect ~~at MD~~, least at LD]).

b) No drug effects on external anomalies of pups, or on necropsy of pups which died or were culled.

c) No drug effects on preweaning development milestones or reflex testing (table 8).

d) No drug effects on postweaning open field behavior, rotarod performance, or T-maze learning. (The latter showed a large degree of inter-animal variation, limiting the power to detect drug effects).

e) No clear drug effects on F_1 reproductive performance. (All selected F allowed to deliver naturally; parameters measured and results shown in tables 21-23. Note, in table 22, that F_2 pup weights tended to be slightly below controls at MD and HD; this may be related to the lower F_1 weights noted above).

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Table 4 Observation on cesarean section of pregnant rats(F₀) treated orally with SDZ ENA 713

Dose (mg/kg/day)	0	0.4	1.3	4
No. of dams examined(F ₀)	25	24	24	24
No. of corpora lutea				
Total	472	441	472	467
Mean ± S.D.	18.9 ± 2.5	18.4 ± 3.0	19.7 ± 3.9	20.3 ± 2.8
No. of implantations				
Total	445	412	394	428
Mean ± S.D.	17.8 ± 2.0	17.2 ± 3.0	16.4 ± 2.4	17.8 ± 4.2
No. of live fetuses				
Total	422	396	379	389
Mean ± S.D.	16.9 ± 2.6	16.5 ± 3.1	15.8 ± 2.3	16.2 ± 4.1
Sex ratio (Male/Female)	221 / 201	212 / 184	210 / 169	191 / 198
Body weight (g)				
Mean ± S.D.	3.07 ± 0.28	3.20 ± 0.14	3.16 ± 0.18	2.87 ± 0.24
Placental weight (mg)				
Mean ± S.D.	434 ± 39	443 ± 38	443 ± 37	404 ± 69
No. of dead fetuses				
Total	23 (5.1 ± 5.9) ^{a)}	16 (3.8 ± 7.3)	15 (3.7 ± 3.6)	39 (9.2 ± 8.8)
Early	16	14	13	31
Late	7	2	2	8

a) : In parentheses, percent of implantations

* : Significantly different from control group at P<0.05

Table 5-1 Effects on fetuses(F₁) of rat dams(F₀) treated orally with SD2 ENA 713

Dose (mg/kg/day)	0	0.4	1.3	4
No. of dams examined(F ₀)	25	24	24	24
No. of fetuses examined	422	396	379	389
No. of external defects	2 ^{b)} (0.8 ± 1.9) ^{c)}	0 (0.0 ± 0.0)	1 ^{d)} (0.3 ± 1.4)	2 ^{e)} (0.5 ± 1.7)
No. of fetuses examined	139	133	125	132
No. of visceral defects	2 ^{f)} (1.4 ± 4.8)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	2 ^{g)} (1.4 ± 4.7)
No. of fetuses examined	283	283	254	257
No. of skeletal defects	1 ^{h)} (0.4 ± 1.8)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)

- a) : Polydactyly of the hindlimb
b) : Agnathia and absence of the tongue
c) : In parentheses, percent of fetuses examined
d) : Vestigial tail
e) : Vestigial tail
f) : General edema and cleft palate
g) : Situs inversus totalis
h) : Unilateral anophthalmia
i) : Absence of the adrenals and ectopia of the kidneys, ovaries and uterus
j) : Abnormal lobation of the lung
k) : Cleft sternbrae
b), h) : The same fetus
e), i) : The same fetus
f), j) : The same fetus

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Table 5-2 Effects on fetuses(F_1) of rat dams(F_0) treated orally with SDZ ENA 713

Dose(mg/kg/day)	0	0.4	1.3	4
No. of dams examined(F_0)	25	24	24	24
No. of skeletal examined	283	263	254	257
Variation				
Total	10 (3.6 \pm 5.9) ^{a)}	16 (5.9 \pm 9.6)	10 (7.2 \pm 10.3)	230 (9.5 \pm 13.7)
Cervical rib	3 ^{b)}	2 ^{d)}	4	8
Lumbar rib	1 ^{c)}	7	8	14
Sacralization of 8th lumbar vertebra	4 ^{b)}	5 ^{d) e)}	2	2
Lumbarization of 1st sacral vertebra	1 ^{c)}	0	0	0
Asymmetry of 13th ribs	3	4 ^{d) e)}	2	1
Hypoplasia of 13th ribs	0	1	0	0
Asymmetry of sternabrae	0	0	1	0

a) : In parentheses, percent of fetuses examined

b)~e) : The same 1 fetus

e : Significantly different from control group at $P < 0.05$

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Table 5-3 Effects on fetuses(F₁) of rat dams(F₀) treated orally with SDZ ENA 713

Dose(ug/kg/day)	0	0.4	1.3	5.0
No. of dams examined(F ₀)	25	24	24	24
No. of skeletal examined	283	263	254	257
Degree of ossification				
Delayed ossification of supraoccipital	2	3	0	1
Splitting of ossification centers of vertebral bodies	0	6*	2	1
Splitting of ossification centers of sternbrae	3	4	3	1
No. of sternbrae	5.4 ± 0.3 ^{a)}	5.6 ± 0.3	5.6 ± 0.4	5.3 ± 0.5
No. of sacro-caudal vertebrae	7.7 ± 0.5	7.6 ± 0.4	7.7 ± 0.5	7.5 ± 0.6
No. of bones in manus				
No. of distal phalanges	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0
No. of middle phalanges	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
No. of proximal phalanges	0.3 ± 0.6	0.2 ± 0.3	0.1 ± 0.2	0.1 ± 0.2
No. of metacarpal	6.8 ± 0.6	7.0 ± 0.5	7.0 ± 0.6	6.5 ± 0.5
No. of bones in pes				
No. of distal phalanges	10.0 ± 0.2	10.0 ± 0.2	10.0 ± 0.1	9.7 ± 1.4
No. of middle phalanges	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
No. of proximal phalanges	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
No. of metatarsal	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	7.9 ± 0.2

a) : Mean ± S.D.

* : Significantly different from control group at P<0.05

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Table 6 Effects of SDZ ENA 713 on gestation, litter size and viability of pups(F₁)

Dose(µg/kg/day)	0	0.4	1.3	4
No. of dams examined(F ₀)	13	13	12	13
No. of dams with live pups	13	13	12	13
Gestation index (%) ^{a)}	100.0	100.0	100.0	100.0
Gestation period (day) Mean ± S.D.	21.7 ± 0.5	21.9 ± 0.3	22.2 ± 0.7	21.8 ± 0.4
At birth				
No. of implantations	218	220	194	221
Mean ± S.D.	16.8 ± 2.4	16.9 ± 3.3	16.2 ± 4.7	17.0 ± 1.6
No. of newborns	195	194	184	204
Mean ± S.D.	15.0 ± 3.9	14.9 ± 4.2	15.3 ± 4.8	15.7 ± 2.2
Delivery index ^{b)}	89.9 ± 16.7	87.0 ± 13.4	91.4 ± 13.7	92.3 ± 10.2
No. of live pups	195 (86 / 109)	194 (94 ^{c)} / 100)	182 (84 / 98)	203 (98 / 105 ^{c)})
Mean ± S.D.	15.0 ± 3.9	14.9 ± 4.2	15.2 ± 4.8	15.6 ± 2.2
Birth index ^{d)}	88.9 ± 16.7	87.0 ± 13.4	90.4 ± 13.7	91.8 ± 10.2
No. of stillbirths	0 (0 / 0)	0 (0 / 0)	2 (2 / 0)	1 (1 / 0)
No. of external defects (%)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	2 ^{ee)} (0.9 ± 2.2)	0 (0.0 ± 0.0)
Lactation period				
No. of perinatal deaths ^{f)}	1 (1 / 0)	6 (5 / 1)	12 ^{g)} (8 / 6)	4 (1 / 3)
Mortality index (%) ^{g)}	0.4 ± 1.5	2.7 ± 4.0	13.2 ± 28.4	1.8 ± 2.9
Mean ± S.D.	1.3 ± 4.6	3.9 ± 6.4	7.3 ± 12.5	1.3 ± 4.6
Viability index on day 4 (%) ^{h)}	99.6 ± 1.3	97.3 ± 4.0	87.8 ± 28.0	98.6 ± 2.6
Mean ± S.D.	98.7 ± 4.6	96.1 ± 6.4	96.4 ± 9.2	100.0 ± 0.0
Weaning index (%) ⁱ⁾	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Mean ± S.D.	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

a) : (No. of females with live pups / No. of pregnant females) × 100

b) : (No. of newborns at birth / No. of implantations) × 100

c) : Including pups died on day 0

d) : (No. of live pups at birth / No. of implantations) × 100

e) : Umbilical hernia

f) : No. of stillbirths + No. of dead pups during 4 days after birth

g) : (No. of perinatal deaths / No. of newborns at birth) × 100

h) : (No. of live pups on day 4 after birth / No. of live pups at birth) × 100

i) : (No. of live pups at weaning / No. of live pups after adjusting on day 4 after birth) × 100

ee) : Significantly different from control group at P<0.01

Table 7 Effects of SDZ ENA 713 on viability and body weight of pups(F₁)

Dose(mg/kg/day)	0	0.4	1.3	4
No. of dams examined(F ₀)	13	13	12 ^a	13
No. of live pups				
At birth Total (Male/Female)	195 (86 / 109)	194 (94 ^a / 100)	182 (84 / 98)	203 (98 / 105 ^a)
On day 4 after birth				
before adjusting Total(M/F)	194 (85 / 109)	188 (89 / 99)	172 ^a (80 / 92)	200 (98 / 102)
after adjusting Total(M/F)	102 (51 / 51)	102 (49 / 53)	88 (42 / 46)	104 (52 / 52)
On day 7 after birth Total(M/F)	102 (51 / 51)	102 (49 / 53)	88 (42 / 46)	104 (52 / 52)
On day 10 after birth Total(M/F)	102 (51 / 51)	102 (49 / 53)	88 (42 / 46)	104 (52 / 52)
On day 14 after birth Total(M/F)	102 (51 / 51)	102 (49 / 53)	88 (42 / 46)	104 (52 / 52)
On day 18 after birth Total(M/F)	102 (51 / 51)	102 (49 / 53)	88 (42 / 46)	104 (52 / 52)
On day 21 after birth Total(M/F)	102 (51 / 51)	102 (49 / 53)	88 (42 / 46)	104 (52 / 52)
Mean body weight of pups (g)				
Male				
At birth	6.5 ± 0.6 ^{b)}	6.7 ± 0.7	6.3 ± 0.5	6.3 ± 0.4
Before adjusting	10.4 ± 1.6	10.7 ± 1.2	9.8 ± 1.3	10.0 ± 1.0
After adjusting	10.4 ± 1.7	10.7 ± 1.3	10.0 ± 1.2	10.0 ± 1.0
On day 7 after birth	17.3 ± 2.6	17.4 ± 1.8	16.0 ± 1.9	17.1 ± 1.4
On day 10 after birth	25.0 ± 3.4	25.5 ± 2.1	24.9 ± 2.1	25.1 ± 2.2
On day 14 after birth	36.3 ± 4.0	37.2 ± 2.5	36.4 ± 2.2	36.5 ± 2.8
On day 18 after birth	46.8 ± 5.2	48.2 ± 3.2	47.9 ± 3.0	47.9 ± 3.5
On day 21 after birth	59.0 ± 6.1	60.7 ± 3.9	60.4 ± 4.5	60.7 ± 4.3
Female				
At birth	6.2 ± 0.8 ^{b)}	6.3 ± 0.6	6.1 ± 0.5	6.0 ± 0.4
Before adjusting	9.9 ± 1.7	10.2 ± 1.2	9.2 ± 1.2	9.5 ± 1.2
After adjusting	10.1 ± 1.7	10.1 ± 1.3	9.2 ± 1.2	9.7 ± 1.2
On day 7 after birth	17.0 ± 2.4	16.6 ± 2.0	15.6 ± 2.2	16.5 ± 1.8
On day 10 after birth	24.9 ± 3.0	24.4 ± 2.6	23.2 ± 2.9	24.3 ± 2.3
On day 14 after birth	36.3 ± 3.4	35.7 ± 3.1	34.3 ± 3.3	35.5 ± 2.8
On day 18 after birth	46.6 ± 4.9	46.6 ± 3.8	45.3 ± 4.0	46.7 ± 3.1
On day 21 after birth	58.0 ± 5.2	58.4 ± 4.3	56.2 ± 5.5	58.7 ± 3.8

a) : Including pups died on day 0

b) : Mean ± S.D.

c) : Excluding the value of 1 dam because of sex misjudgment.

d) : Significantly different from control group at P<0.05

Table 8 Effects of SDZ ENA 713 on postnatal development and reflex functions in rat pups(P_1) of the treated dams(P_0)

Dose(mg/kg/day)	0	0.4	1.3	4
No. of dams examined(P_0)	13	13	11 ^{a)}	13
Postnatal physical development				
Separation of ear auricle (on day 4)	102 / 102 ^{b)}	102 / 102	88 / 88	104 / 104
Appearance of dorsal hair (on day 4)	102 / 102	102 / 102	88 / 88	104 / 104
Eruption of upper incisors (on day 14)	102 / 102	102 / 102	88 / 88	104 / 104
Separation of eyelids (on day 17)	102 / 102	102 / 102	88 / 88	104 / 104
Descent of testes (day, Mean \pm S.D.)	23.8 \pm 0.7 (13) ^{c)}	24.1 \pm 1.1 (13)	24.5 \pm 0.7 (11)	24.5 \pm 1.0 (13)
Opening of vagina (day, Mean \pm S.D.)	31.9 \pm 1.7 (13)	31.2 \pm 2.3 (13)	31.5 \pm 1.6 (11)	31.3 \pm 2.0 (13)
Reflex functions				
Preyer reflex (on day 18)	102 / 102 ^{d)}	102 / 102	88 / 88	104 / 104
Pain reflex (on day 19)	102 / 102	102 / 102	88 / 88	104 / 104
Righting reflex (on day 20)	102 / 102	102 / 102	88 / 88	104 / 104
Righting in mid-air (on day 20)	102 / 102	102 / 102	88 / 88	104 / 104
Corneal reflex (on day 21)	102 / 102	102 / 102	88 / 88	104 / 104
Pupillary reflex (on the 3rd week)	26 / 26	26 / 26	22 / 22	26 / 26

a) : The value of 1 animal was excluded from mean calculation because all pup of the animal died.

b) : No. of developing pups / No. of pups examined

c) : In parentheses, No. of pups examined

d) : No. of pups with normal reflex / No. of pups examined

Table 21 Effects of SDZ ENA 713 on gestation, litter size and viability of pups (F₂)

Dose (ng/kg/day)	0	0.4	1.3	4
No. of dams examined (F ₁)	10	10	8	11
Gestation period (day) Mean ± S.D.	21.9 ± 0.3 ^{a)}	21.9 ± 0.3	21.6 ± 0.3	22.0 ± 0.0
At birth				
No. of implantations Total	152	151	105	180
Mean ± S.D.	15.2 ± 3.2	15.1 ± 3.0	13.1 ± 4.4	16.4 ± 2.4
No. of newborns Total	137	138	98	161
Mean ± S.D.	13.7 ± 3.6	13.8 ± 3.6	12.3 ± 4.4	14.6 ± 2.8
Delivery index ^{b)} Mean ± S.D.	89.1 ± 9.2	90.6 ± 9.8	92.0 ± 7.5	89.5 ± 10.5
No. of live pups Total (Male/Female)	134 (66 / 68)	137 (69 / 68)	98 (50 ^{c)} / 48)	160 (77 / 83)
Mean ± S.D.	13.4 ± 3.4	13.7 ± 3.5	12.3 ± 4.4	14.5 ± 2.9
Birth index ^{d)} Mean ± S.D.	87.5 ± 10.4	90.1 ± 9.4	92.0 ± 7.5	88.8 ± 10.6
No. of stillbirths Total (Male/Female)	3 (1 / 2)	1 (0 / 1)	0 (0 / 0)	1 (1 / 0)
No. of external defects (%)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)
Lactation period				
No. of perinatal deaths ^{e)} Total (Male/Female)	4 (1 / 3)	3 (2 / 1)	4 (3 / 1)	7 (5 / 2)
Mortality index (%) ^{f)} Total	2.4 ± 7.4	2.0 ± 3.2	5.1 ± 9.9	5.2 ± 13.8
Mean ± S.D. Male	1.3 ± 4.0	3.0 ± 8.4	6.4 ± 8.9	7.5 ± 16.8
Female	3.3 ± 10.5	1.3 ± 4.0	7.1 ± 18.9	4.5 ± 15.1
Viability index on day 4 (%) ^{g)} Total	99.3 ± 2.3	98.6 ± 3.0	94.9 ± 9.9	95.3 ± 12.2
Mean ± S.D. Male	100.0 ± 0.0	98.3 ± 8.4	93.6 ± 8.9	93.3 ± 14.9
Female	98.6 ± 4.5	100.0 ± 0.0	92.9 ± 18.9	95.5 ± 15.1
Weaning index (%) ^{h)} Total	100.0 ± 0.0	100.0 ± 0.0	92.2 ± 22.1	100.0 ± 0.0
Mean ± S.D. Male	100.0 ± 0.0	100.0 ± 0.0	93.8 ± 17.7	100.0 ± 0.0
Female	100.0 ± 0.0	100.0 ± 0.0	89.3 ± 28.3	100.0 ± 0.0

a) : The value of 1 animal was excluded from mean calculation because uncertainty of copulation data.

b) : (No. of newborns at birth / No. of implantations) × 100

c) : Including pups died on day 0

d) : (No. of live pups at birth / No. of implantations) × 100

e) : No. of stillbirths + No. of dead pups during 4 days after birth

f) : (No. of perinatal deaths / No. of newborns at birth) × 100

g) : (No. of live pups on day 4 after birth / No. of live pups at birth) × 100

h) : (No. of live pups at weaning / No. of live pups after adjusting on day 4 after birth) × 100

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Table 22 Effects of SDZ ENA 713 on viability and body weight of pups (P₂)

Dose(mg/kg/day)	0	0.4	1.3	4
No. of dams examined(P ₂)	10	10	8	11
No. of live pups				
At birth Total (Male/Female)	134 (66 / 68)	137 (69 / 68)	98 (50 ^a) / 48)	160 (77 / 83)
On day 4 after birth before adjusting Total(M/F)	133 (66 / 67)	135 (67 / 68)	94 (47 / 47)	154 (73 / 81)
after adjusting Total(M/F)	78 (39 / 39)	80 (39 / 41)	57 (32 / 25)	86 (42 / 44)
On day 7 after birth Total(M/F)	78 (39 / 39)	80 (39 / 41)	53 (31 / 22)	86 (42 / 44)
On day 14 after birth Total(M/F)	78 (39 / 39)	80 (39 / 41)	52 ^a (30 / 22)	86 (42 / 44)
On day 21 after birth Total(M/F)	78 (39 / 39)	80 (39 / 41)	52 ^a (30 / 22)	86 (42 / 44)
Mean body weight of pups (g)				
Male				
At birth	6.8 ± 0.9 ^{b)}	6.9 ± 0.7 ^{a)}	6.8 ± 0.7	6.7 ± 0.7
Before adjusting	11.4 ± 2.4	11.5 ± 1.4	11.3 ± 1.8	10.2 ± 1.6
After adjusting	11.4 ± 2.4	11.6 ± 1.4	11.5 ± 1.7	10.2 ± 1.9
On day 7 after birth	18.3 ± 2.3	18.8 ± 1.5	17.6 ± 3.8	16.6 ± 2.6
On day 14 after birth	26.8 ± 3.3	27.3 ± 2.7	25.6 ± 7.9	25.6 ± 2.6
On day 21 after birth	31.1 ± 5.4	30.1 ± 4.3	26.0 ± 10.6	28.1 ± 4.9
Female				
At birth	6.5 ± 0.7	6.6 ± 0.6 ^{a)}	6.1 ± 0.6	6.4 ± 0.6
Before adjusting	10.9 ± 2.2	11.0 ± 1.2	10.3 ± 1.3	9.7 ± 1.8
After adjusting	11.0 ± 2.2	11.1 ± 1.2	10.2 ± 1.3	9.9 ± 1.7
On day 7 after birth	17.7 ± 2.3	17.9 ± 1.1	15.7 ± 3.2	16.6 ± 2.2
On day 14 after birth	25.8 ± 3.0	25.9 ± 1.7	22.4 ± 6.3	25.3 ± 2.1
On day 21 after birth	28.0 ± 4.3	27.3 ± 2.8	22.1 ± 7.9	27.5 ± 3.9

a) : Including 2 pups died on day 0

b) : Mean ± S.D.

c) : Excluding the value of 2 dams because of sex misjudgment

d) : Significantly different from control group at P<0.05