6 MONTH CARCINOGENICITY IN P 53 TRANSGENIC MOUSE

A) Dosage

15/sex at 0, 2.5, 5, 10, or 20 mg/kg/day, by gavage.

Lab performing study:
Department of Toxicology
Janssen Research Foundation
2340 Beerse, Belgium

Drug batch #: MR113675PFP091

Strain: SPF deficient (heterozygous), supplied by

B) Results

1) Observed signs

a) Sedation

Slight sedation at M-HD, and moderate sedation at HD, seen throughout study. Severe sedation seen at both of these doses during the first 2 weeks.

b) Tremors

Seen at M-HD, mainly first week, and at HD "almost during the entire study".

2) Mortality

8 of 15 HD M died on first day of dosing. Aside from this, there were deaths in 1 MD M (week 12), an additional HD M (week 14), 2 M-HD F (week 19 and 21), and 3 HD F (weeks 5,7, and 7); however, except for possibly in one of the HD F, none of the deaths occurring after day 1 were considered drug-related with most presumed to be dosing accidents.

Note that in a rangefinding study using the originating wild type strain, no deaths were seen at the highest dose of 20 mg/kg given for 1 month. (N=5/sex).

3) Bodyweight gain

Decreased in all groups (but equivocal and not statically significant in LD F). Not strongly D-R.

Final weight as % of control:

	M	F
LD	91	96
MD	85	93
M-HD	82	89
HD	85	89

See attached figure.

4) Food Consumption

Sporadic decreases in all groups but LD M, not strongly D-R.

5) Hematology
(Performed week 26)

a) RBC, Hb, Hct

Very slight, non D-R decreases in all M groups. (No clear effect in F; Hb and Hct were very slightly decreased at LD and MD only).

b) WBC

Total WBC slightly decreased in all M groups except LD; numbers of all individual cell types also slightly decreased.

c) Other parameters measured: platelets, normoblasts

6) Blood chemistry
(Performed at termination)

No clear/ pronounced effects. Potassium was slightly increased in all M groups, partly D-R. Glucose was decreased in MD and HD (but not M-HD) M; mean at MD and HD was 84% and 73% of control, respectively.

Other parameters measured: Na, Cl, Ca, inorganic P, total protein, albumin, cholesterol, triglycerides, phospholipids, BUN, creatinine, total bilirubin, AP, AST, ALT.

7) Organ weights

No effects not likely secondary to decreased bodyweights.

8) Gross pathology

No drug effects

9) Histopathology

(Routine exam done in all groups. List of organs examined is attached).

a) Neoplastic changes

Only a few tumors were seen, with no relation to drug. (See attached tables)

b) Non-neoplastic changes

No clear drug effects. A possible slight decrease in incidence and severity in chronic inflammation of salivary gland seen in HD M at terminal sacrifice.

10) Plasma drug levels

Samples taken at termination, at 1, 2, 4, 6 and 10 hours post dose (except in HD M: 1, 4, and 10 hours only). N=2/ time point.

Results shown in attached table. AUCs increased somewhat greater than in proportion to dose, and were about 2x greater in M than in F.

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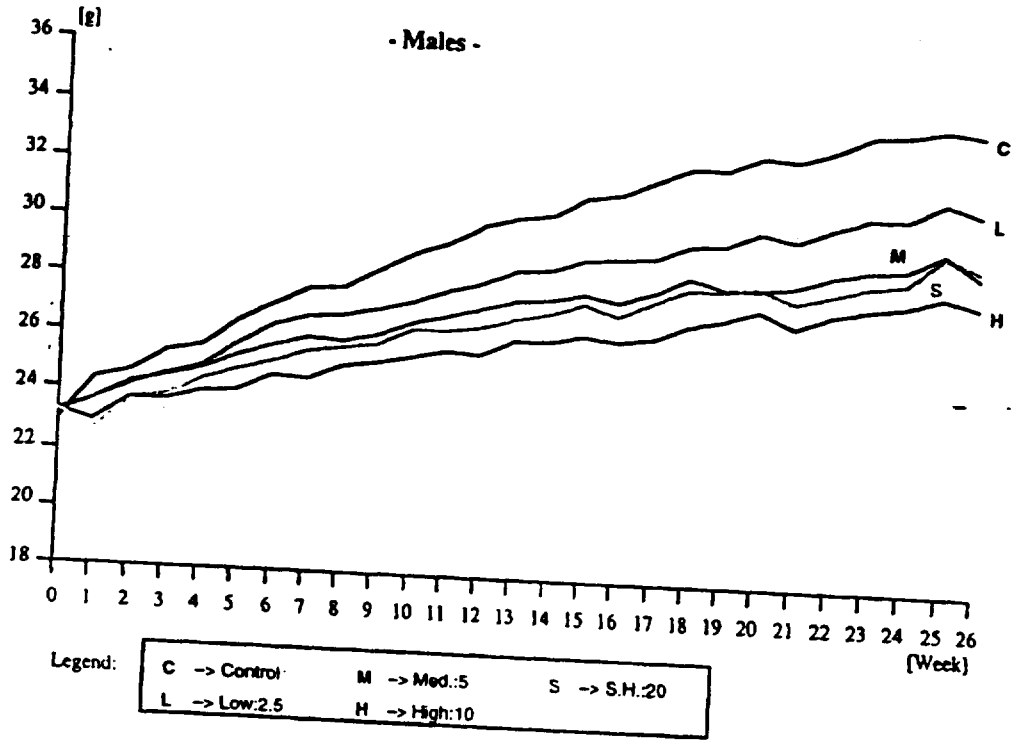
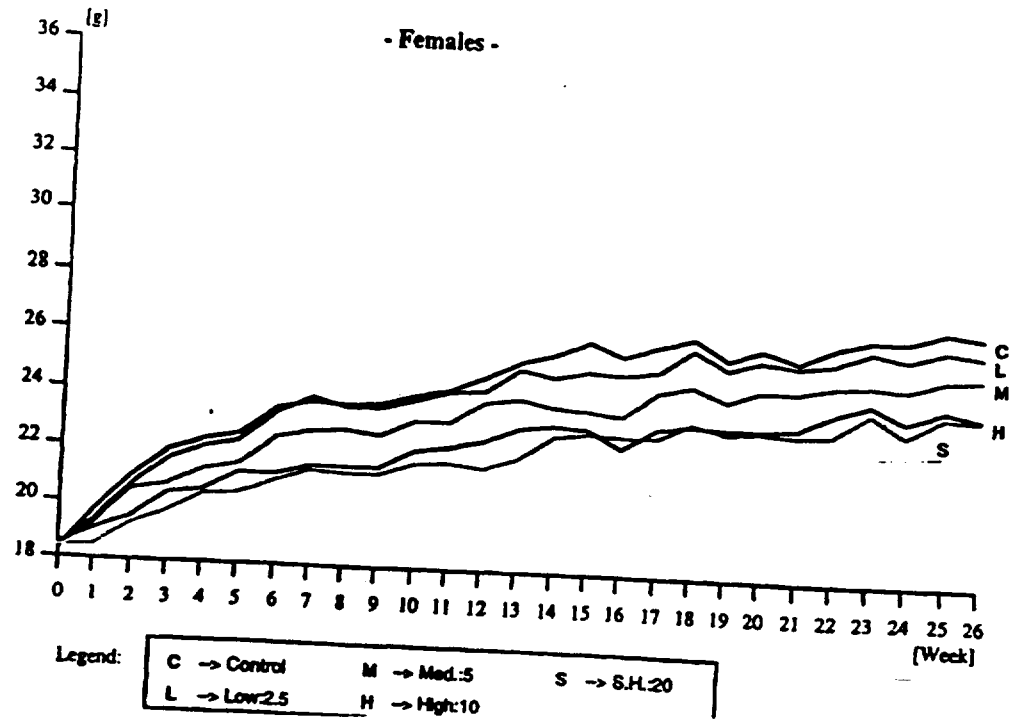
Experiment: 4451
6-Month Carcinogenicity Study
R113675 - OR/GAV - MOUSE

BODY WEIGHT
Mean values per dosage group in g





2.4.5. HISTOPATHOLOGY

The mice of this experiment were examined histologically after routine sampling and processing of the tissues. Standardised methods were used to obtain comparable specimens.

The following tissues were examined in all animals:

- adrenal glands
- aorta
- bone marrow
- bone, sternum
- bone, stifle joint
- brain
- coagulating glands
- epididymides
- esophagus
- extraorbital lacrimal gland
- eye
- gall bladder
- heart
- kidneys
- large intestines (cecum, colon, rectum)
- liver
- lungs
- lymph node(s), mesenteric
- mammary gland
- nose
- ovaries
- pancreas
- parathyroid gland(s)
- peripheral nerve, sciatic nerve
- pituitary gland
- prostate
- salivary gland, submandibular
- seminal vesicles
- skeletal muscle, psoas muscle
- skin
- small intestines (duodenum, jejunum, ileum)
- spinal cord, thoracic
- spleen
- stomach, forestomach
- stomach, glandular stomach
- testes
- thymus
- thyroid glands
- trachea
- urinary bladder
- uterus
- vagina
- gross lesions

The tissues of control and dosed mice were prepared for histological examination by

Standardised methods were used to obtain comparable specimens. The , was used.

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EXPERIMENT: 4451
6-Month Carcinogenicity Study
R113675 - OR/GAV - MOUSE

HISTOPATHOLOGY

Tumor incidences per dosage group

MALES

Organ or Tissue - Observation	Dosage group (mg / kg)				
	Control	Low:2.5	Med.:5	High:10	S.High:20
Number examined:	*15*	*15*	*15*	*15*	*15*
Lymphoid and hematopoietic system					
- Lymphosarcoma			1		
- Lymphosarcoma, lymphocytic	1				
- Thymoma, predominantly lymphocytic, malignant			1		
Soft tissue *Number examined:*	*15*	*15*	*15*	*15*	*15*
- Hemangioma			1		

Significance versus Control computed by the Fisher Exact test (one tailed) : * P < .05 ** P < .01 *** P < .001
Statistics are only performed if more than 50 % of the animals of the group are examined

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EXPERIMENT: 4451
6-Month Carcinogenicity Study
R113675 - OR/GAV - MOUSE

HISTOPATHOLOGY

Tumor incidences per dosage group

FEMALES

Organ or Tissue - Observation		Dosage group (mg / kg)				
		Control	Low:2.5	Med.:5	High:10	S.High:20
Bone	*Number examined:*	*15*	*15*	*15*	*15*	*15*
- Osteosarcoma		1				
	Number examined:					
Lymphoid and hematopoietic system		*15*	*15*	*15*	*15*	*15*
- Lymphoid leukemia		1				
- Lymphosarcoma, lymphocytic						1

Significance versus Control computed by the Fisher Exact test (one tailed) : * P < .05 ** P < .01 *** P < .001
Statistics are only performed if more than 50 % of the animals of the group are examined

Table 5-3: **Mean (n = 2) serum concentrations (ng/ml) and some pharmacokinetic parameters of galantamine in TSG-P53 transgenic mice measured on day 182 of a 6-month oral (gavage) carcinogenicity study on aqueous solutions of galantamine hydrobromide (R113675) at 2.5, 5, 10 and 20 mg base-eq./kg/day (Exp. No. 4451).**

	2.5 mg/kg/day		5 mg/kg/day		10 mg/kg/day		20 mg/kg/day	
Time (h)	Males	Females	Males	Females	Males	Females	Males	Females
1	98	70	267	150	395	223	1341	845
2	36	15	71	48	281	199		328
4	12	< 10	45	13	106	38	402	163
6	< 10	< 12	15	10	36	18		49
10	< 12	< 13	< 10	< 10	< 10	< 10	20	17[1]
C_{max} (ng/ml)	98	70	267	150	395	223	1341	845
T_{max} (h)	1	1	1	1	1	1	1	1
$t_{1/2}$[2] (h)	1.3	-[3]	1.3	1.8	1.3	1.9	1.5	1.9
AUC_{0-t} (ng.h/ml)	164	78	479	258	1065	616	4101	1844
$AUC_{0-24\,h}$ (ng.h/ml)	187	117[4]	507	284	1132	665	4144	1891

1) n = 1
2) The half-lives are calculated with the concentrations in the shaded areas.
3) Not calculated.
4) For the calculation of the $AUC_{0-24\,h}$, the mean of the $t_{1/2}$ of the 5- (1.8 h), 10- (1.9 h) and 20-mg/kg (1.9 h) dose levels was used.

ONE YEAR P.O. TOXICITY IN BEAGLE DOGS:

A) Dosage

11/ sex at 0, 8/ sex at 1.6 and 4, and 11/ sex at 8 mg/ kg/ day, in capsules.

4/ sex/ group were sacrificed at 6 and 12 months. The remaining 3/ sex in controls and HD were kept for a 4 week recovery period.

Study performed by:
Drug lot #: "CDF 2612 and CDF 2643"

B) Results

1) Observed signs

a) All doses: tremors, fasciculations, salivation, lacrimation, diarrhea, mucoid/ soft feces, hyperactivity. (All D-R)

b) MD and HD only: emesis

c) HD only: low incidence of ataxia, excessive panting, and hyperactivity

d) "Most, if not all" signs said to be reversible

2) Mortality

No drug effects. (One control M was sacrificed for humane reasons week 26).

3) Bodyweight gain

No drug effects aside from a transient, slightly increased weight loss/ decreased gain in some HD M near the beginning of the study.

4) Food Consumption

Text states that there were no drug effects on "estimated" food consumption but no data were shown.

5) Physical/auditory (handclap)/ophthalmoscopic/ EKG exams.

(Performed pre-study and months 3, 6, 9, and 12, and [except ophthalmoscopy] after recovery period. EKG recordings were taken approximately 4-6 hr. post-dose).

There were no clearly drug-related effects, aside from observed signs noted above. EKG exams showed ST depression in 1 HD M on day 168. It was not seen at repeat exams on days 177 and 180. No elevations of AST or ALT were seen in this animal when measured on day 181. This animal also had "no correlative heart histopathological findings to indicate an effect on the heart". In addition to this dog, an HD F had "occasional" SA block on day 78, and an MD F had "occasional" 2° A-V block on day 167; these were not considered to be drug related.

6) Hematology

(Done pre-study, months 3, 6, 9, and 12, and after recovery period).

No clear drug effects. RBC, Hb, and Hct were sporadically very slightly increased, primarily at HD.

Other parameters measured: reticulocytes, platelets, Heinz bodies, PT, WBC, differential

7) Blood chemistry

(Done pre-study, months 3, 6, 9, and 12, and after recovery).

No clear drug effects. A few very slight sporadic differences from control were noted, including increases in glucose and decreases in P and K, which are only noted here because more pronounced and clearly drug-related changes were seen in the 1 year rat study.

There was no demonstrable inhibition of "cholinesterase" in serum, RBC, or brain.

Other parameters measured: ALT, AST, AP, GGT, LDH, total bilirubin, cholesterol, triglycerides, total protein, albumin, creatinine, BUN, Ca, Na, and Cl.

8) Urinalysis
(Done pre-study months 3, 6, 9, and 12, and after recovery period).

No drug effects.

Parameters measured: volume, SG, pH, protein, glucose, ketones, bilirubin, urobilinogen, occult blood, cells, casts, crystals.

9) Organ weights

No clear drug effects, although slight drug effects would be difficult to determine due to the low Ns, and large variability for some organs. Absolute and relative testes weights were slightly below controls in all M groups at termination, but not D-R. Absolute and relative thymus weights were slightly increased in HD F at the 6 month and terminal sacrifices.

10) Gross Pathology

No summary tables given. Text states that at terminal sacrifice, drug-related effects at HD "included distension of the uterus with fluid (nonrecovery) and a tissue mass in the uterus (recovery)", and that "both changes were diagnosed microscpically as pseudocyesis and endometrial hyperplasia." (See below).

11) Microscopic pathology

(Routine exam done in all groups, except only selected organs in recovery groups).

a) Urinary bladder

Increased incidences of focal or multifocal degeneration of the tunica muscularis at HD. In HD M, incidence at 6 months was 3/4 (vs 0/5 controls); however at 12 months the incidence was not increased (1/4 vs 1/3 controls). In HD F the incidence at 6 months was 1/4 (vs 0/4 controls) and at 12 months was 2/4 (vs 0/4 controls). It was stated that in HD F, the severity of the lesion was less at 12 than at 6 months. The lesion was not seen in recovery dogs.

b) Uterus/ ovaries

Increased incidence of pseudopregnancy associated with endometrial hyperplasia and a slight increase in the size and/ or number of ovarian corpora lutea, seen at 12 months in 1/4 MD F and 2/4 HD F. Also seen in 1/3 recovery HD F (0/3 in controls; other groups not examined).

12) Plasma levels of parent drug

Levels were measured, in dogs scheduled to be sacrificed at 1 year, just prior to dosing and at 2, 6, and 24 hours post-dose during weeks 4 and 50. Results shown in attached tables. Note that most values were BLQ (< 125 ng/ml) except for the 2 hour timepoint (all doses) and 6 hour time point (HD only). (Compound not detected in pre-dosing or 24 hr. samples; not shown in table). Results at the 2 hour time point show that levels were roughly proportional to dose and were similar at 4 and 50 weeks. There were no consistent sex differences.

Table 1

Concentrations of galanthamine in the plasma of dogs in week 4 of a Toxicology 26/52-week oral toxicity study of galanthamine HBr (MIN 921004)

Animal Number	Sex	Concentration of Galanthamine (ng/ml)[a] 2 hr	Mean ± Std. Dev.	6 hr	Mean ± Std. Dev.
2 mg/kg/day					
16	M	191		BLQ[b]	
17		198		BLQ	
18		162	175 ± 24	BLQ	—[c]
19		148		BLQ	
54	F	256		BLQ	
55		288		BLQ	
56		259	260 ± 21	BLQ	—
57		238		BLQ	
6 mg/kg/day					
24	M	BLQ		N.S.[d]	
25		218		BLQ	
26		361	324 ± 301	BLQ	—
27		716		BLQ	
62	F	179		289	
63		441		BLQ	
64		694	459 ± 214	BLQ	—
65		522		BLQ	
10 mg/kg/day					
32	M	850		491	
33		979		449	
34		854	888 ± 61	327	444 ± 82
35		869		510	
70	F	1555		179	
71		1580		144	
72		1440	1521 ± 62	184	159 ± 27
73		1508		130	

[a] Concentrations in pre-dose and 24-hour samples were all below the limit of quantification (125 ng/ml).
[b] BLQ = below the limit of quantification.
[c] No value is given if the calculated mean (using BLQ = 0) was less than the limit of quantification.
[d] N.S. = no sample.

Table 2

Concentrations of galanthamine in the plasma of dogs in week 50 of a Toxicology 26/52-week oral toxicity study of galanthamine HBr (MIN 921004)

Animal Number	Sex	Concentration of Galanthamine (ng/ml)[a] 2 hr	Mean ± Std. Dev.	6 hr	Mean ± Std. Dev.
2 mg/kg/day					
16	M	232		BLQ[b]	
17		196		BLQ	
18		294	233 ± 43	BLQ	—[c]
19		210		BLQ	
54	F	137		BLQ	
55		276		BLQ	
56		207	220 ± 63	BLQ	—
57		262		BLQ	
5 mg/kg/day					
24	M	755		177	
25		251		266	
26		354	475 ± 222	BLQ	—
27		539		BLQ	
62	F	387		BLQ	
63		493		BLQ	
64		425	474 ± 89	BLQ	—
65		590		BLQ	
10 mg/kg/day					
32	M	1634		282	
33		1330		258	
34		1138	1311 ± 233	220	190 ± 129
35		1142		BLQ	
70	F	1463		171	
71		1088		126	
72		705	992 ± 361	151	—
73		713		BLQ	

[a] Concentrations in pre-dose and 24-hour samples were all below the limit of quantification (125 ng/ml).
[b] BLQ = below the limit of quantification.
[c] No value is given if the calculated mean (using BLQ = 0) was less than the limit of quantification.

FERTILITY AND EMBROYNIC DEVELOPMENT STUDY IN RATS

A) Dosage / Methods

25 / sex at 0, 2, 8, or 16 mg/kg/day, by gavage, from day 60 pre-mating through the 10 day mating period (M) or from day 14 pre-mating through day 17 of pregnancy (F).

M were sacrificed after mating period; sperm number, motility, and morphology were evaluated. F were sacrificied day 20 of pregnancy; half of live fetuses examined for visceral findings by microdissection and sectioning, and remaining fetuses examined for skeletal findings with Alizarin red S staining.

Strain: Sprague-Dawley derived rats , supplied by

Lab Where Study Performed:

Drug Batch #: 22205

B) Results

1) Observed signs

Tremors at MD and HD, salivation at HD, and noisy breathing during first few days of treatment in HD M. (In a rangefinding study, 32 mg/kg caused severe clinical signs).

2) Mortality

1 HD M died on day 75 of treatment. It was stated that "there were no previous abnormal clinical signs but on day 75 it was observed with brown furstaining and post-dose salivation." (In the rangefinding study 32 mg/kg caused convulsions death in 1 of 6 M).

3) Bodyweight

a) M: D-R decreased gain at MD and HD. Weights at end of dosing period 7 and 12 % below control, respectively. (See attached figure).

b) F: During pre-mating period, slight bodyweight loss at HD, and slightly decreased weight gain at MD and (not statistically significant) LD. During gestation, decreased weight gain at MD and HD. Mean weight at end of dosing period 93 and 90% of control, respectively. (See attached figure; the days pre-mating given on the abscissa are apparently in error).

4) Food consumption

Decreased at MD and HD. (Means at HD ~ 85-90% of control).

5) Mating performance/fertility

Slight (13%) decrease in number of estrous cycles during the pre-mating dosing period at HD. No drug effects on mating or fertility.

No drug effect on sperm analysis.

6) Pregnancy data

No drug effects on numbers of CL, implantations, resorptions, or live and dead fetuses, on fetal sex ratio, on pre- or post-implantations loss, or on fetal weight.

7) Fetal exams

No drug effects on external or visceral exams.

Drug - related increases in minor skeletal abnormalities were seen at MD and HD. (Sponsor's summary table 16, showing detailed results of skeletal exam is attached; following this is table 14, showing the overall results of external, visceral and skeletal exams; the second page of table 14 gives overall incidence values for skeletal abnormalities). The primary minor skeletal abnormalities increased by drug were "one or more sternebrae bilobed, bipartitie, misshapen or misaligned"; fetal incidence was 7%, 7%, 18%, and 16% in C, LD, MD, and HD, respectively. (Sponsor's historical control mean given as 1.6%, with a range of %). The sponsor also notes an increase in wavy ribs (0, 0.6, 1.6, and 2.3% of fetuses in C, LD, MD, and HD, resp.), although these values were within the sponsor's historical control range ( ). The incidence of total minor skeletal abnormalities at MD and HD (24% of fetuses) was above the sponsors historical range ( ).

The incidence of the skeletal variant vestigial 14th rib was increased at HD (fetal incidence 18.5% vs 8.0% controls; smaller non-statistically significant increases also seen at LD and MD; see table); however the incidences were within the historical range ( ).

8) Plasma drug levels

Levels of galantamine and norgalantamine were measured in a rangefinding study (volume 1.59, p.46) in which rats were given galantamine from 2 weeks pre-mating for 5 weeks (M) or through day 17 of pregnancy (F). Levels were measured on the first and last days of these treatment periods. Doses and results are shown in the attached tables. (Note that AUC $_{0.25\text{-}8\ hours}$ was calculated; as shown in the tables levels were still relatively high in some cases at 8 hours). Galantamine AUC increased roughly in proportion to dose at the second time point, but somewhat less than proportionally on day 1. Galantamine AUCs at the second time point were somewhat greater than those on day 1 (up to ~ 2x). Galantamine AUCs in F were ~ 1.5-2x those in M. Levels of norgalantamine were much lower than those of galantamine.

Figure 1

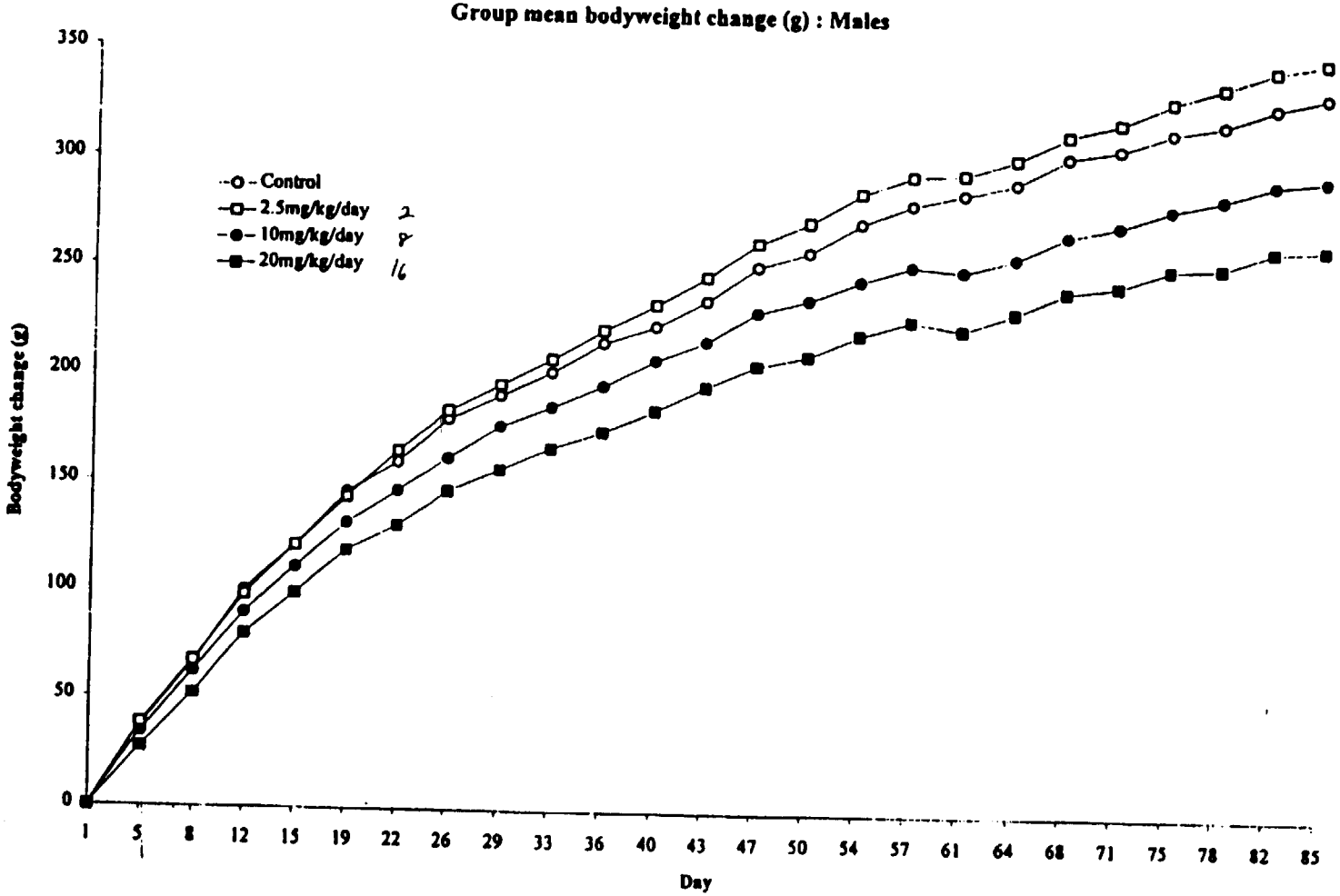
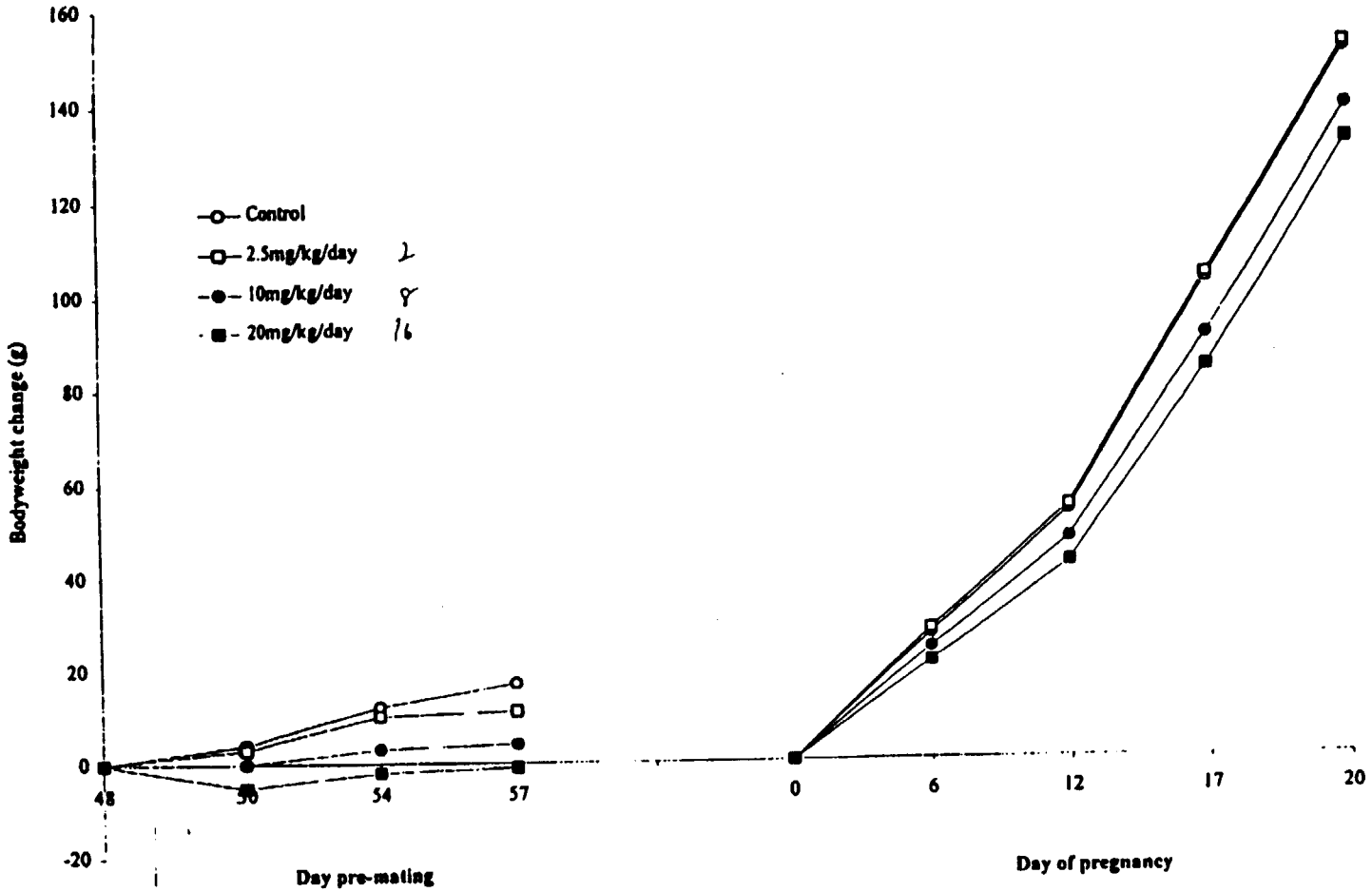

Group mean bodyweight change (g) : Males
Bodyweight change (g)
Day
Control
2.5mg/kg/day
10mg/kg/day
20mg/kg/day
0
50
100
150
200
250
300
350
1
5
8
12
15
19
22
26
29
33
36
40
43
47
50
54
57
61
64
68
71
75
78
82
85

Figure 2

Group mean bodyweight change (g) : Females



Study number : SPD/017/R - Rat Fertility and Embryonic Development Study

TABLE 16

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Group	:	1	2	3	4
Test article	:	Control		Galanthamine	
Dose level (mg/kg/day)	:	0	~~2.5~~ 2	~~10~~ 8	~~20~~ 16

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4	
	Total number of foetuses examined		167		171		186		172	
	Total number of litters examined		24		24		25		24	
	Skull									
b	Frontal: Incomplete ossification	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.5)
c	One or more: Fissure/plaque of bone integral to normal structure of bone	Variant	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)
d	Parietal: Incomplete ossification	Variant	5	(2.8)	6	(3.7)	2	(1.1)	11	(5.5)
e	Interparietal: Incomplete ossification	Variant	19	(10.4)	19	(10.9)	13	(6.9)	24	(12.2)
f	Occipital: Incomplete ossification	Variant	5	(2.8)	6	(3.4)	5	(2.9)	10	(4.9)
g	Hyoid: Not ossified	Variant	0	(0.0)	1	(0.5)	0	(0.0)	2	(1.2)
	Cervical vertebra									
h	One or more centra: Ossified	Variant	120	(72.3)	124	(73.0)	97	(53.4)	112	(65.2)
	Thoracic vertebra									
7	Number of vertebra: 14	Minor	3	(1.8)	1	(0.6)	0	(0.0)	3	(1.8)
8	One or more centra: Not ossified	Minor	1	(0.6)	0	(0.0)	0	(0.0)	1	(0.7)
i	One or more centra: Incomplete ossification	Variant	2	(1.1)	1	(0.6)	4	(2.0)	1	(0.5)

Study Number : SPD/017/R - Rat Fertility and Embryonic Development Study

TABLE 16 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4	
Total number of foetuses examined			167		171		186		172	
Total number of litters examined			24		24		25		24	
Thoracic vertebra (continued)										
9	One or more centra: Hemicentric	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)
10	One or more centra: Asymmetrically ossified	Minor	2	(1.0)	1	(0.6)	3	(1.7)	1	(0.5)
)	One or more centra: Bilobed	Variant	22	(12.3)	22	(14.3)	38	(20.4)	22	(12.6)
11	One or more centra: Bipartite	Minor	1	(0.6)	0	(0.0)	0	(0.0)	1	(0.5)
Lumbar vertebra										
12	Number of vertebra: 5	Minor	2	(1.2)	1	(0.6)	0	(0.0)	2	(1.2)
13	Number of vertebra: 7	Minor	1	(0.6)	0	(0.0)	1	(0.4)	0	(0.0)
14	One or more centra: Bilobed	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)
15	One or more centra: Not ossified	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)
16	One or more neural arch: Incomplete ossification	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.5)
Sacral vertebra										
17	One or more centra: Not ossified	Minor	1	(0.6)	0	(0.0)	0	(0.0)	1	(0.7)
18	One or more neural arch: Incomplete ossification	Minor	2	(1.1)	6	(3.6)	3	(1.6)	5	(2.6)

Study number : SPD/017/R - Rat Fertility and Embryonic Development Study

TABLE 16 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		
	Total number of foetuses examined		167		171		186		172		
	Total number of litters examined		24		24		25		24		
	Sacral vertebra (continued)										
19	One or more neural arch: Not ossified	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	
	Caudal vertebra										
20	Number of centra: <=2	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	
21	Number of neural arches: 0	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	
	Rib										
22	Rib- Uni- or bilateral: Cervical	Minor	0	(0.0)	1	(0.5)	0	(0.0)	3	(2.0)	T*
23	One or more: Incomplete ossification	Minor	0	(0.0)	0	(0.0)	1	(0.5)	4	(1.9)	
D	One or more: Absent	Major	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)	
24	One or more: Wavy	Minor	0	(0.0)	1	(0.6)	3	(1.6)	5	(2.3)	
k	14th- Uni- or bilateral: Vestigial	Variant	15	(8.0)	25	(16.8)	24	(12.8)	31*	(18.5)	T*
25	14th- Uni- or bilateral: Extra	Minor	3	(1.8)	1	(0.6)	0	(0.0)	3	(1.8)	
E	One or more: Arising from same neural arch	Major	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)	
	Sternum										
26	1st sternebra: Not ossified	Minor	1	(0.6)	0	(0.0)	0	(0.0)	1	(0.7)	

T - Dose response * - p<0.05 ** - p<0.01 *** - p<0.001

Study number : SPD/017/R - Rat Fertility and Embryonic Development Study

TABLE 16 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		
Total number of foetuses examined			167		171		186		172		
Total number of litters examined			24		24		25		24		
Sternum (continued)											
27	1st sternebra: Incomplete ossification	Minor	1	(0.6)	0	(0.0)	2	(1.1)	0	(0.0)	
28	2nd sternebra: Not ossified	Minor	2	(1.2)	0	(0.0)	0	(0.0)	1	(0.7)	
29	2nd sternebra: Incomplete ossification	Minor	3	(1.6)	3	(1.9)	6	(3.3)	6	(3.6)	
30	3rd sternebra: Not ossified	Minor	1	(0.6)	0	(0.0)	0	(0.0)	1	(0.7)	
31	3rd sternebra: Incomplete ossification	Minor	1	(0.6)	0	(0.0)	2	(0.8)	0	(0.0)	
32	4th sternebra: Not ossified	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	
33	4th sternebra: Incomplete ossification	Minor	1	(0.6)	0	(0.0)	4	(1.8)	1	(0.7)	
34	One or more: Bilobed, bipartite, mis-shapen or misaligned	Minor	12	(6.9)	12	(6.6)	35**	(17.6)	26**	(15.5)	T***
m	5th sternebra: Not ossified	Variant	2	(1.0)	4	(2.2)	3	(1.3)	6	(3.7)	
n	6th sternebra: Not ossified	Variant	2	(1.2)	0	(0.0)	1	(0.5)	1	(0.7)	
Pelvic girdle											
35	Ischium- Uni- or bilateral: Incomplete ossification	Minor	1	(0.6)	0	(0.0)	0	(0.0)	1	(0.7)	
36	Pubis- Uni- or bilateral: Not ossified	Minor	1	(0.6)	0	(0.0)	0	(0.0)	1	(0.7)	

T - Dose response * - p<0.05 ** - p<0.01 *** - p<0.001

Study number : SPD/017/R - Rat Fertility and Embryonic Development Study

TABLE 16 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		
Total number of foetuses examined			167		171		186		172		
Total number of litters examined			24		24		25		24		
Forelimb											
37	Humerus- Uni- or bilateral: Incomplete ossification	Minor	0	(0.0)	1	(0.6)	0	(0.0)	1	(0.5)	
o	One or more metacarpal: Not ossified	Variant	12	(6.6)	14	(7.6)	11	(5.4)	2	(1.2)	
p	One or more phalange: Ossified	Variant	115	(64.5)	121	(68.7)	136	(72.9)	145	(83.9)	T**
Hindlimb											
38	One or more metatarsal: Not ossified	Minor	1	(0.6)	0	(0.0)	1	(0.5)	1	(0.7)	
q	One or more phalange: Ossified	Variant	1	(0.5)	0	(0.0)	0	(0.0)	1	(0.6)	

T - Dose response * - p<0.05 ** - p<0.01 *** - p<0.001

Study number : SPD/017/R - Rat Fertility and Embryonic Development Study

TABLE 14

Foetal examination : summary of group mean data

Group	:	1	2	3	4
Test article	:	Control		Galanthamine	
Dose level (mg/kg/day)	:	0	2 ~~2.5~~	8 ~~10~~	16 ~~20~~

	Group 1	Group 2	Group 3	Group 4
External and visceral examination				
Total number of litters examined	24	24	25	24
Total number of foetuses examined	334	341	371	342
Number with major abnormalities	1	0	2	0
Mean % of foetuses examined	0.3	0.0	0.5	0.0
Number of litters affected	1	0	2	0
Number with minor abnormalities	2	1	1	2
Mean % of foetuses affected	0.6	0.3	0.2	0.6
Number of litters affected	2	1	1	2
Number with variations	99	76	62	68
Mean % of foetuses affected	28.0	21.6	16.5	19.4
Number of litters affected	18	19	19	19

Study number : SFD/017/R - Rat Fertility and Embryonic Development Study

TABLE 14 (continued)

Foetal examination : Summary of group mean data

	Group 1	Group 2	Group 3	Group 4	
Skeletal examination					
Total number of litters examined	24	24	25	24	
Total number of foetuses examined	167	171	186	172	
Number with major abnormalities	0	0	0	1	
Mean % of foetuses examined	0.0	0.0	0.0	0.6	
Number of litters affected	0	0	0	1	
Number with minor abnormalities	23	22	47	42	
Mean % of foetuses affected	12.9	12.7	24.1	24.0	
Number of litters affected	13	14	18	21 *	T**
Number with variations	155	170	171	169	
Mean % of foetuses affected	91.7	99.4	91.9	97.9	
Number of litters affected	24	24	25	24	

T - Dose response * - $p<0.05$ ** - $p<0.01$ *** - $p<0.001$

Study number : SPD/017/R - Rat Fertility and Embryonic Development Study

TABLE 14 (continued)

Foetal examination : summary of group mean data

	Group 1	Group 2	Group 3	Group 4
Combined examination (external/visceral/skeletal)				
Total number of litters examined	24	24	25	24
Total number of foetuses examined	334	341	371	342
Number with major abnormalities	1	0	2	1
Mean % of foetuses examined	0.3	0.0	0.5	0.3
Number of litters affected	1	0	2	1

MALES

TABLE 4: Plasma concentrations (ng/ml) and some pharmacokinetic parameters of galantamine and of norgalantamine measured in pooled plasma samples (n = 3) obtained after single (day 1) and repeated (day 36) administration of aqueous solutions of galantamine hydrobromide (R113675) at 2.5, 10, 20 and 40 mg/kg/day in male rats in an oral (gavage) general reproductive performance dose ranging study (

	galantamine							
	2 ~~2.5~~ mg/kg		8 ~~10~~ mg/kg		16 ~~20~~ mg/kg		32 ~~40~~ mg/kg	
Time (h) after dosing	Day 1	Day 36	Day 1	Day 36	Day 1	Day 36	Day 1	Day 36
0	≤ 10	-1)	≤ 10	-1)	≤ 10	-1)	≤ 10	-1)
0.25	107	119	294	370	497	998	766	-1)
0.5	135	134	640	640	426	1085	500	-1)
1	114	136	493	579	399	975	430	-1)
2	88	76	281	321	352	614	606	-1)
4	26	46	116	220	242	486	328	-1)
8	≤ 10	13	88.9	78.0	257	199	333	-1)
C_{max} (ng/ml)	135	136	640	640	497	1085	766	-2)
T_{max} (h)	0.50	1.0	0.50	0.50	0.25	0.50	0.25	-2)
$AUC_{0.25\text{-}8\ h}$ (ng.h/ml)	360 3)	445	1594	2018	2289	4040	3165	-2)
	norgalantamine							
	2 ~~2.5~~ mg/kg		8 ~~10~~ mg/kg		16 ~~20~~ mg/kg		32 ~~40~~ mg/kg	
Time (h) after dosing	Day 1	Day 36	Day 1	Day 36	Day 1	Day 36	Day 1	Day 36
0	≤ 25	-2)	≤ 25	-1)	≤ 25	-1)	≤ 25	-1)
0.25	≤ 25	29	51	52	76	107	89	-1)
0.5	31	≤ 25	63	64	48	128	54	-1)
1	≤ 25	36	68	92	42	120	75	-1)
2	≤ 25	≤ 25	40	61	49	94	≤ 100	-1)
4	≤ 25	≤ 25	36	67	38	92	56	-1)
8	≤ 25	≤ 25	46	≤ 25	44	76	78	-1)
C_{max} (ng/ml)	31	36	68	92	76	128	89	-2)
T_{max} (h)	0.5	1	1.0	1.0	0.25	0.50	0.25	-2)
$AUC_{0.25\text{-}8\ h}$ (ng.h/ml)	-2)	-2)	341	258 4)	335	720	514	-2)

1) sample not collected.
2) parameter not calculated or determined.
3) $AUC_{4\text{-}8\ h}$ calculated from $C_{4\ h/\beta}$ (β was calculated by log linear regression of the plasma concentration time profile).

Females

TABLE 5: Plasma concentrations (ng/ml) and some pharmacokinetic parameters of galantamine and of norgalantamine measured in pooled plasma samples (n = 3) obtained after single (day 1) and repeated (day 17 of pregnancy) administration of aqueous solutions of galantamine hydrobromide (R113675) at 2.5, 10 and 20 mg/kg/day in female rats in an oral (gavage) general reproductive performance dose ranging study (Study Number: SPD/16/R of Quintiles/Toxicol.).

	galantamine					
	2 ~~2.5~~ mg/kg		~~8~~ 10 mg/kg		16 ~~20~~ mg/kg	
Time (h) after dosing	Day 1	Day 17 [1]	Day 1	Day 17 [1]	Day 1	Day 17 [1]
0	≤ 10	-[2]	≤ 10	-[2]	≤ 10	-[2]
0.25	199	203	1021	713	931	1142 [3]
0.5	214	318	502	738 [3]	469	1295
1	174	269	500 [3]	789 [4]	490	1265
2	101	175	483	597	672	1151
4	38	80	226	442	210	706
8	19	27	175	205	272	389
C_{max} (ng/ml)	214	318	1021	789	931	1295
T_{max} (h)	0.50	0.50	0.25	1.0	0.25	0.50
$AUC_{0.25\text{-}8\ h}$ (ng.h/ml)	539	903	2443	3589	2842	6200
	norgalantamine					
	2 ~~2.5~~ mg/kg		~~8~~ 10 mg/kg		16 ~~20~~ mg/kg	
Time (h) after dosing	Day 1	Day 17 [1]	Day 1	Day 17 [1]	Day 1	Day 17 [1]
0	≤ 25	-[2]	≤ 25	-[2]	≤ 25	-[2]
0.25	≤ 25	≤ 25	53	33	47	48 [3]
0.5	≤ 25	≤ 25	31	54 [3]	36	55
1	≤ 25	≤ 25	28	52 [4]	28	81
2	≤ 25	≤ 25	36	54	47	114
4	≤ 25	≤ 25	34	36	25	67
8	≤ 25	≤ 25	-[5]	≤ 25	29	32
C_{max} (ng/ml)	-[6]	-[6]	53	54	47	114
T_{max} (h)	-[6]	-[6]	0.25	0.50	0.25	2.0
$AUC_{0.25\text{-}8\ h}$ (ng.h/ml)	-[6]	-[6]	127 [7]	180 [7]	244	523

[1] corresponds with day 17 of pregnancy.
[2] sample not collected.
[3] n = 2.
[4] n = 1.
[5] interfering peaks.
[6] parameter not calculated or determined.
[7] $AUC_{0.25\text{-}4\ h}$.

PRE- AND POST-NATAL DEVELOPMENT STUDY IN RATS

A) Dosage / Methods

25 pregnant F at 0, 2, 8, or 16 mg/ kg/ day, by gavage, from day 6 of pregnancy through day 20 PP.

Dams were allowed to deliver and rear their young. Dams sacrificed day 21 PP; 20/ sex F_1 pups per group were retained for developmental and fertility assessment.

Strain: Sprague Dawley supplied by

Lab where study was performed:

Drug Batch #: 22205

B) Results

1) Observed signs in Fo dams

Tremors and post-dose salivation toward the end of lactation period were seen in a few HD. One HD F was hypersensitive to external stimuli and convulsed at the end of the lactation period. No effect at LD or MD.

2) Mortality of Fo dams

No drug effects. (One control was sacrificed in mid-parturition).

3) Fo bodyweight

Decreased weight gain between days 6 and 20 of pregnancy at MD and HD. (Weights on day 20 were 96% and 93% of control, resp.). Weight gain also decreased at HD between days 1 and 7 PP. At day 21 PP, weights were below controls at MD and HD (97% and 93% of control, resp.). See attached figure.

4) Fo food consumption

Decreased at MD and HD throughout the dosing period. The magnitude of effect decreased over time; mean values were about 85% - 95% of control at MD and 80-90% of control at HD.

5) Fo reproductive results

No drug effects on duration of gestation, mean number of pups born (total or alive), pup sex ratio, pup ano-genital distance, and pup survival.

Pup weights were decreased through day 21 PP at MD and HD. (Mean pup weights on day 21 PP ~93% and 90% of control at MD and HD, resp). (See attached table "6").

6) F_1 pup developmental observations

No drug effects on the following: Pre-weaning: % with ears open day 4, % with righting reflex day 5, % with startle response day 15, % with eyes open day 15 (a large decrease was seen at MD, but only a slight decrease at HD), and % with pupillary light reflex on day 21. Post-weaning: E maze learning test (days 28-29 PP), ophthalmoscopic exam (days 28-35 PP), auditory startle response (days 28-35 PP), day of preputial separation and vaginal perforation.

F_1 bodyweights (which were below controls at MD and HD through day 21 PP as noted above) remained below controls through week 17 PP in MD and HD M; weight gain weeks 4-17 PP was slightly decreased in these groups. (Week 17 weights in MD and HD M 95% and 92% of control, resp.). In contrast, MD and HD F_1 pups gained slightly more than controls during the post-weaning period, such that by week 7 PP weights were the same as controls.

There were no drug-related effects on necropsy of pups which died prematurely or were killed at culling on day 4 PP or on day 21 PP

7) F_1 reproductive performance

No drug effects on mating, fertility, or reproductive parameters (mean numbers of CL, implantations, live embryos [there was a slight statistically significant increase in the latter 2 at HD], and pre- and post - implantation loss) measured on day 13 of gestation.

TABLE 6

Group mean pup bodyweights and bodyweight gains (g) ± S.D. : F1 generation litters

Group	1	2	3	4
Treatment	Control		Galanthamine	
Dosage (mg/kg/day)	0	~~25~~ 2	~~10~~ 8	~~20~~ 16

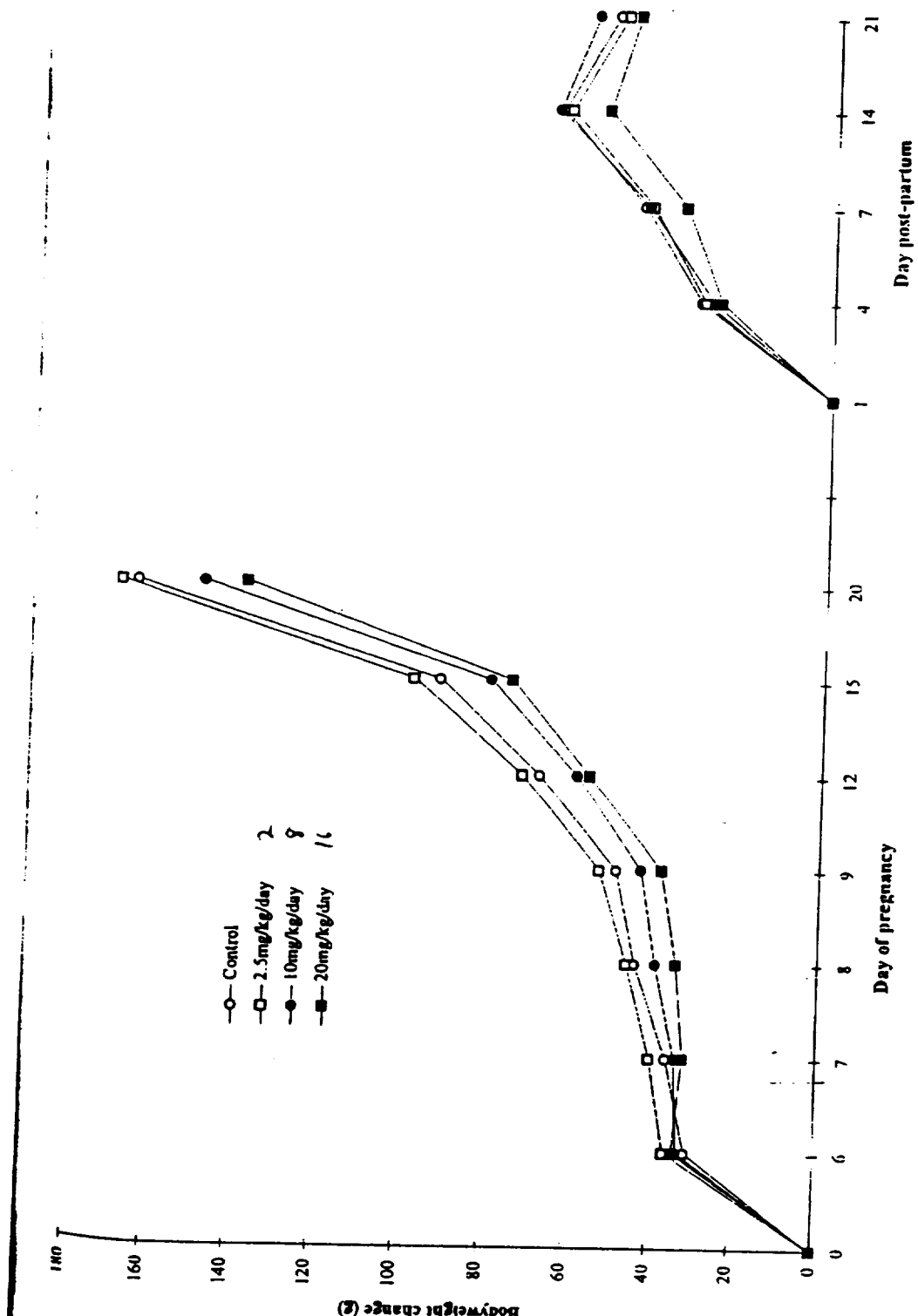
Males

Bodyweight (day post partum)	Group 1	2	3	4	Analysis of variance
N	20	22	24	24	
0	6.2 ± 0.3	6.2 ± 0.3	6.0 ± 0.4	5.9 ± 0.4**	p<0.05
4	9.1 ± 0.7	9.1 ± 1.0	8.8 ± 1.0	8.6 ± 1.1	NS
4pc	9.1 ± 0.7	9.2 ± 0.9	8.8 ± 1.1	8.6 ± 1.0	NS
7	15.3 ± 1.3	15.3 ± 1.6	14.3 ± 2.0*	13.8 ± 1.7**	p<0.01
14	33.1 ± 2.7	33.0 ± 2.8	31.1 ± 3.7*	29.3 ± 2.6***	p<0.001
21	53.8 ± 4.6	53.7 ± 4.1	50.6 ± 5.5* 94%	48.7 ± 5.1** 91%	p<0.01
Bodyweight gain (days 0 - 21)	47.5 ± 4.6	47.6 ± 4.0	45.4 ± 5.6	42.9 ± 5.0**	p<0.01

Females

Bodyweight (day post partum)	Group 1	2	3	4	Analysis of variance
N	20	22	24	24	
0	5.9 ± 0.3	5.8 ± 0.3	5.7 ± 0.3	5.6 ± 0.4**	p<0.05
4	8.7 ± 0.8	8.7 ± 0.9	8.3 ± 0.9	8.3 ± 1.1	NS
4pc	8.8 ± 0.8	8.7 ± 0.9	8.3 ± 0.9	8.3 ± 1.1	NS
7	14.8 ± 1.3	14.5 ± 1.6	13.8 ± 1.8*	13.3 ± 1.6**	p<0.01
14	32.4 ± 2.4	31.7 ± 2.5	30.1 ± 3.3**	28.4 ± 2.4***	p<0.001
21	53.1 ± 4.5	52.0 ± 3.7	49.6 ± 5.4** 93%	47.8 ± 4.7*** 90%	p<0.001
Bodyweight gain (days 0 - 21)	47.1 ± 4.5	46.2 ± 3.6	43.8 ± 5.3*	42.2 ± 4.4***	p<0.01

N = number of litters in mean
pc = post culling
* = significantly different from controls, p<0.05, Williams' test
** = significantly different from controls, p<0.01, Williams' test
*** = significantly different from controls, p<0.001, Williams' test


Bodyweight change (g)
0
20
40
60
80
100
120
140
160
Day of pregnancy
1
6
7
8
9
12
15
20
Day post-partum
1
4
7
14
21
Control
2.5mg/kg/day
10mg/kg/day
20mg/kg/day

EMBRYONIC DEVELOPMENT STUDY IN RABBITS

A) Dosage / Methods

20 F at 0, 4, 12, 28, or 40 mg/kg/day, by gavage, days 6-18 of pregnancy.

Dams sacrificed day 28 of pregnancy. Fetuses examined for external, visceral, and skeletal abnormalities by standard methods.

Strain: New Zealand White

Lab Where Study Performed:

Drug Batch #: 22205

B) Results

1) Observed signs

a) No effects at LD and MD
b) Trembling seen in 3 M-HD
c) Aggressiveness, excessive feet stamping, tremors, and increased incidence of abnormal fecal output (reduced quantity/liquid/loose/absent) at HD

d) (In rangefinding studies, doses up to 32 mg/kg/day in pregnant rabbits, and up to 48 mg/kg/day in non-pregnant rabbits, were tested. Pronounced observed signs were not seen; tremors were seen in 2 of 4 rabbits at 48 mg/kg).

2) Mortality

a) 1 HD sacrificed due to abnormal signs on first day of dosing, including trembling, noisy and rapid breathing, constricted pupils, and convulsions.

b) 1 rabbit each at MD, M-HD, and HD aborted and were sacrificed prematurely.

3) Bodyweights

M-HD and HD had mean weight loss on first 2 days of dosing, followed by decreased gain. Mean weight on day 18 of pregnancy was 95% and 93 % of control at M-HD and HD, resp. Some rebound seen after cessation of dosing. (See attached figure).

4) Food consumption

Decreased at M-HD and HD, D-R. At HD, mean consumption during treatment period ~ 75% of control.

5) Pregnancy data

No drug effects on numbers of resorptions or live or dead fetuses, post-implantation loss, fetal sex ratio, or fetal weight.

6) Fetal Exams

Summary tables attached.

A slightly higher % of fetuses with major external/visceral abnormalities was seen at HD (table 7); however the types of abnormalities seen were varied across fetuses (table 10) and incidence values were said to be WNL.

There was a slight/equivocal increase in the minor skeletal abnormalities "cornua of the hyroid bone bent " and incomplete ossification of maxilla at HD (Table 11). (The former was within the given historical range; a range for the latter was not given).

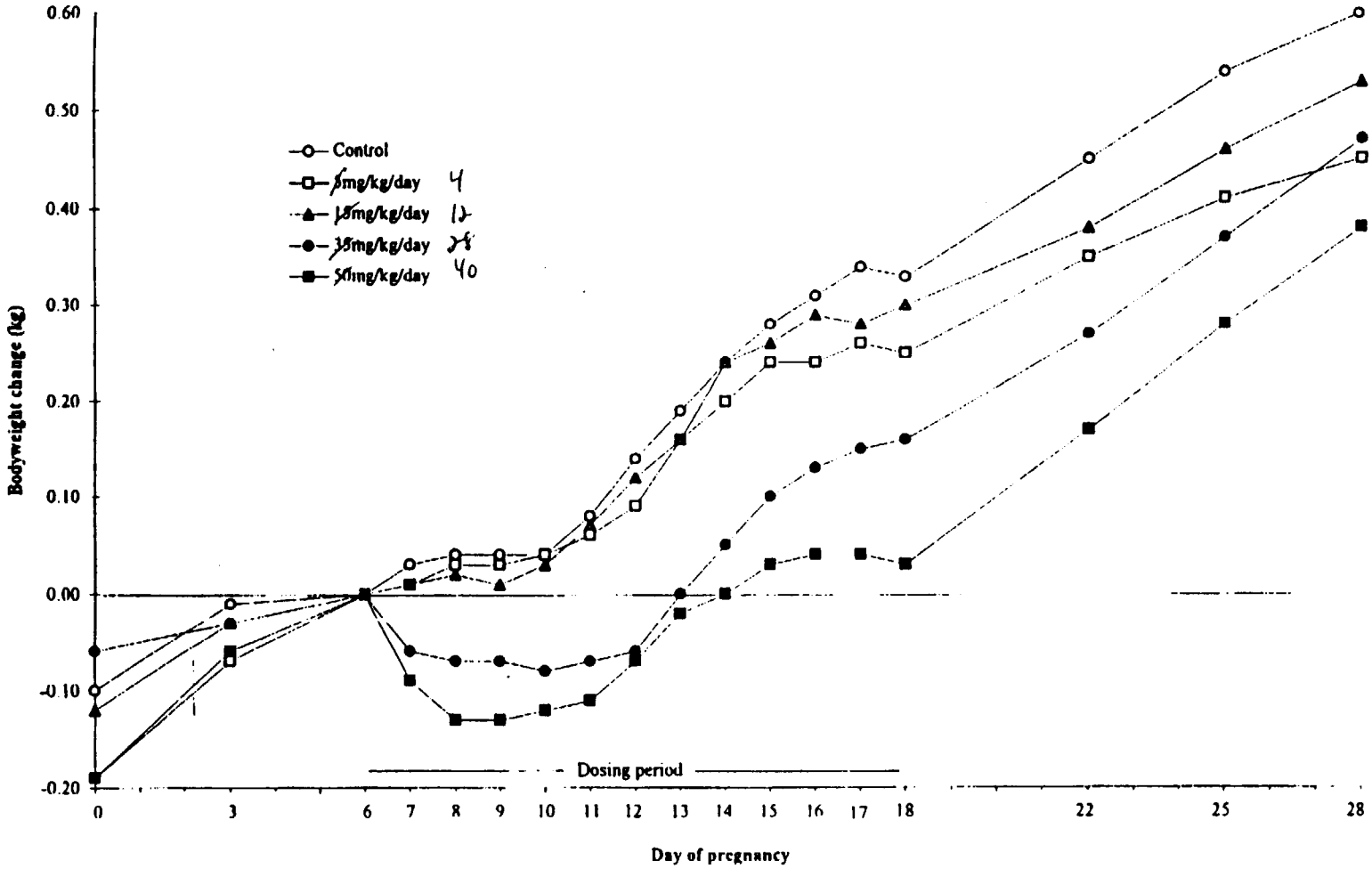
Several skeletal variants had a higher incidence in all drug groups; however these were not D-R and said to be WNL. These included 13 thoracic vertebrae, 6 lumbar vertebrae, extra 13th rib, and incomplete ossification of 1 or more phalanges of fore - and hind limbs. (Table 11).

7) Plasma drug levels

Levels of galantamine and norgalantamine were measured in a rangefinding study in which New Zealand White rabbits were given galantamine from days 6-18 of pregnancy; levels were measured on days 6 and 18. Doses and results shown in attached tables. The small Ns and large inter-animal variation make conclusions difficult. Galantamine AUCs on day 18 were roughly proportional to dose. Galantamine AUCs on day 18 were 2-4 x those on day 6. Levels of norgalantamine were generally below the level of quantification.

Figure 1

Group mean maternal bodyweight change (kg)



38a

Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 7

Foetal examination : summary of group mean data

Group	:	1	2	3	4	5
Test article	:	Control		Galanthamine		
Dose level (mg/kg/day)	:	0	~~4~~	~~15~~ 12	~~30~~ 28	~~50~~ 40

	Group 1	Group 2	Group 3	Group 4	Group 5
External and visceral examination					
Total number of litters examined	16	16	18	17	18
Total number of foetuses examined	152	133	180	153	158
Number with major abnormalities	1	3	2	2	5
Mean % of foetuses examined	0.5	1.9	1.1	1.2	3.3
Number of litters affected	1	3	2	2	5
Number with minor abnormalities	35	27	30	18	24
Mean % of foetuses affected	22.3	19.4	19.6	11.5	15.9
Number of litters affected	13	12	12	10	13
Number with variations	0	0	0	0	0
Mean % of foetuses affected	0.0	0.0	0.0	0.0	0.0
Number of litters affected	0	0	0	0	0

Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 8

Foetal examination : summary of group mean data

Group	:	1	2	3	4	5
Test article	:	Control		Galanthamine		
Dose level (mg/kg/day)	:	0	~~5~~ 4	~~10~~ 12	~~30~~ 28	~~50~~ 40

	Group 1	Group 2	Group 3	Group 4	Group 5
Skeletal examination					
Total number of litters examined	16	16	18	17	18
Total number of foetuses examined	152	133	180	153	158
Number with major abnormalities	2	1	3	3	1
Mean % of foetuses examined	1.1	0.6	1.8	1.8	0.9
Number of litters affected	1	1	2	2	1
Number with minor abnormalities	25	23	33	19	44
Mean % of foetuses affected	14.9	16.2	19.8	12.1	24.8
Number of litters affected	9	11	14	12	14
Number with variations	142	132	177	146	154
Mean % of foetuses affected	92.8	99.1	98.1	94.4	97.3
Number of litters affected	16	16	18	17	18

Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 9

Foetal examination : summary of group mean data

Group	:	1	2	3	4	5
Test article	:	Control		Galanthamine		
Dose level (mg/kg/day)	:	0	~~8~~ 4	~~15~~ 12	~~35~~ 28	~~50~~ 40

	Group 1	Group 2	Group 3	Group 4	Group 5
Combined examination (external/visceral/skeletal)					
Total number of litters examined	16	16	18	17	18
Total number of foetuses examined	152	133	180	153	158
Number with major abnormalities	3	4	4	4	5
Mean % of foetuses examined	1.7	2.5	2.2	2.4	3.3
Number of litters affected	2	4	3	3	5

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Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 10

External and visceral examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Group	:	1	2	3	4	5
Test article	:	Control		Galanthamine		
Dose level (mg/kg/day)	:	0	5~~4~~	15~~12~~	35~~28~~	50~~40~~

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		Group 5	
	Total number of foetuses examined		152		133		180		153		158	
	Total number of litters examined		16		16		18		17		18	
	Thoracic cavity											
1	Subclavian artery- Right: Retro-oesophageal	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.7)	0	(0.0)
2	Common carotid artery- Left: Abnormal, arising from innominate artery	Minor	18	(10.0)	21	(15.1)	20	(11.5)	7	(4.6)	14	(10.0)
A	Aortic arch: Interrupted	Major	0	(0.0)	1	(0.8)	0	(0.0)	0	(0.0)	0	(0.0)
B	Aortic arch: Enlarged	Major	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.7)
C	Aortic arch: Constricted	Major	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.7)	0	(0.0)
D	Aortic arch: Pre-ductal coarctation	Major	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.9)
E	Aortic arch: Retro-oesophageal	Major	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.7)	0	(0.0)
3	Aortic arch: Additional blood vessel	Minor	18	(12.8)	6	(4.4)	8	(7.1)	9	(5.4)	12	(8.3)
F	Pulmonary arch: Constricted	Major	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.7)
G	Aortic and pulmonary arch: Aortic valvular atresia	Major	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)	1	(0.6)
H	Aortic and pulmonary arch: Pulmonary valvular atresia	Major	0	(0.0)	2	(1.1)	1	(0.4)	0	(0.0)	1	(0.6)
I	Aortic and pulmonary arch: Persistent truncus arteriosus	Major	1	(0.5)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
J	Ductus arteriosus: Constricted	Major	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.7)
4	Ventricle- Uni- or bilateral: Mis-shapen	Minor	0	(0.0)	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)
5	Central post caval lung lobe: Agenesis	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(1.0)	0	(0.0)

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Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 10 (continued)

External and visceral examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key Finding	Type	Group 1	Group 2	Group 3	Group 4	Group 5
Total number of foetuses examined		152	133	180	153	158
Total number of litters examined		16	16	18	17	18
Abdominal cavity						
6 Gall bladder: Bilobed	Minor	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
7 Gall bladder: Duplicated	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
Spine						
K Cervical, thoracic, lumber or sacral cord: Spina bifida	Major	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)
Umbilicus						
L Umbilicus: Umbilical hernia	Major	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Body						
8 Entire: Runted foetus	Minor	0 (0.0)	1 (0.6)	3 (1.5)	0 (0.0)	2 (1.0)

Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 11

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Group	:	1	2	3	4	5
Test article	:	Control		Galanthamine		
Dose level (mg/kg/day)	:	0	4	12	28	40

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		Group 5	
	Total number of foetuses examined		152		133		180		153		158	
	Total number of litters examined		16		16		18		17		18	
	Skull											
9	Fontanelle- Anterior: Increased in size	Minor	1	(0.5)	2	(1.1)	4	(3.5)	0	(0.0)	1	(0.9)
10	Fontanelle- Posterior: Increased in size	Minor	0	(0.0)	1	(0.6)	1	(1.4)	0	(0.0)	1	(0.9)
11	Frontal- Suture: Premature sutural closure	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.7)	0	(0.0)
a	One or more: Fissure/plaque of bone integral to normal structure of bone	Variant	3	(1.9)	3	(2.2)	7	(3.7)	4	(3.1)	9	(5.2)
12	Parietal: Incomplete ossification	Minor	3	(1.9)	2	(1.2)	1	(0.7)	2	(1.5)	4	(2.6)
13	Parietal- Suture: Premature sutural closure	Minor	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	2	(1.0)
14	Interparietal: Incomplete ossification	Minor	0	(0.0)	1	(0.6)	2	(2.1)	0	(0.0)	0	(0.0)
15	Interparietal: Bipartite	Minor	0	(0.0)	4	(2.5)	0	(0.0)	0	(0.0)	0	(0.0)
16	Maxilla- Uni- or bilateral: Incomplete ossification	Minor	0	(0.0)	3	(2.1)	0	(0.0)	0	(0.0)	6	(3.0)
17	Hyoid: Cornua bent	Minor	0	(0.0)	2	(1.6)	3	(3.2)	2	(1.0)	7*	(4.2) T**
18	Zygomatic arch and maxilla- Uni- or bilateral: Premature partial fusion	Minor	16	(9.2)	5	(3.9)	8	(3.5)	7	(4.7)	3	(1.7)

from 2 litters
from 6 litters

T - trend test * - p<0.05 ** - p<0.01 *** - p<0.001

Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 11 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		Group 5	
Total number of foetuses examined			152		133		180		153		158	
Total number of litters examined			16		16		18		17		18	
Cervical vertebra												
19	One or more centra: Bipartite	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)	0	(0.0)
20	One or more centra: Mis-shapen	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)	0	(0.0)
21	One or more centra: Asymmetrically ossified	Minor	1	(0.6)	1	(0.8)	0	(0.0)	2	(1.5)	2	(1.2)
22	One or more centra: Incomplete ossification	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(1.0)	0	(0.0)
23	One or more centra: Bilobed	Minor	0	(0.0)	0	(0.0)	1	(0.6)	1	(0.6)	0	(0.0)
N	One or more neural arch: Absent	Major	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)	0	(0.0)
24	One or more neural arch: Mis-shapen	Minor	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)	0	(0.0)
Cervical, thoracic, lumbar, sacral or caudal vertebra												
N	One or more: Scoliosis	Major	1	(0.6)	0	(0.0)	0	(0.0)	1	(0.6)	0	(0.0)
Thoracic vertebra												
b	Number of vertebra: 13	Variant	30	(18.4)	54	(39.1)	75	(39.7)	76	(51.6)	63	(39.6)
25	One or more centra: Misplaced	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
26	One or more centra: Asymmetrically ossified	Minor	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.7)

Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 11 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		Group 5	
	Total number of foetuses examined		152		133		180		153		158	
	Total number of litters examined		16		16		18		17		18	
	Thoracic vertebra (continued)											
27	One or more centra: Hemicentric	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
28	One or more centra: Bilobed	Minor	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
0	One or more neural arch: Absent	Major	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Cervical, thoracic, lumbar, sacral or caudal vertebra											
P	One or more: Spina bifida	Major	0	(0.0)	0	(0.0)	1	(0.7)	0	(0.0)	0	(0.0)
	Lumbar vertebra											
c	Number of vertebra: 6	Variant	17	(9.8)	30	(19.8)	46	(24.4)	26	(17.6)	28	(18.5)
d	Number of vertebra: 8	Variant	4	(2.9)	3	(1.9)	5	(4.1)	6	(4.0)	3	(1.8)
	Caudal vertebra											
e	Number of centra: <=14	Variant	21	(13.3)	27	(20.5)	27	(15.0)	18	(11.3)	28	(16.7)
f	Number of neural arches: <=6	Variant	13	(8.5)	21	(15.8)	17	(10.1)	17	(11.1)	24	(13.9)
29	One or more centra: Offset	Minor	0	(0.0)	1	(0.8)	0	(0.0)	0	(0.0)	0	(0.0)
	Rib											
30	Rib: Uni- or bilateral: Cervical	Minor	1	(0.6)	0	(0.0)	1	(1.4)	0	(0.0)	1	(0.5)

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TABLE 11 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1	Group 2	Group 3	Group 4	Group 5
Total number of foetuses examined			152	133	180	153	158
Total number of litters examined			16	16	18	17	18
Rib (continued)							
Q	One or more: Absent	Major	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
R	One or more: Fused	Major	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
31	One or more: Bifurcated	Minor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
g	13th- Uni- or bilateral: Vestigial	Variant	24 (15.4)	45 (36.6)	36 (19.4)	38 (26.0)	29 (18.2)
h	13th- Uni- or bilateral: Floating	Variant	3 (2.0)	6 (4.6)	6 (3.6)	2 (1.6)	2 (1.6)
i	13th- Uni- or bilateral: Extra	Variant	31 (19.2)	55 (39.8)	77 (40.8)	78 (53.0)	66 (41.4)
Sternum							
32	Additional centre- One or more: Ossified	Minor	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
33	1st sternebra: Incomplete ossification	Minor	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
34	One or more: Mis-shapen or misaligned	Minor	2 (1.1)	4 (2.8)	5 (3.8)	4 (2.5)	3 (2.0)
S	One or more: Fused	Major	0 (0.0)	1 (0.6)	2 (1.1)	1 (0.7)	1 (0.9)
35	3rd sternebra: Incomplete ossification	Minor	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
36	4th sternebra: Incomplete ossification	Minor	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
37	One or more: Bilobed or bipartite (sternebra 1-4)	Minor	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)

Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 11 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		Group 5	
	Total number of foetuses examined		152		133		180		153		158	
	Total number of litters examined		16		16		18		17		18	
	Sternum (continued)											
38	One or more: Fused	Minor	0	(0.0)	2	(1.4)	2	(1.3)	4	(2.6)	0	(0.0)
j	5th sternebra: Not ossified	Variant	25	(14.8)	21	(15.0)	40	(20.7)	28	(17.5)	38	(25.1)
k	5th sternebra: Incomplete ossification	Variant	51	(33.9)	52	(37.5)	57	(30.5)	35	(21.3)	41	(25.0)
m	One or more: Bilobed or bipartite (sternebra 5+6)	Variant	0	(0.0)	1	(0.8)	0	(0.0)	0	(0.0)	0	(0.0)
n	6th sternebra: Not ossified	Variant	8	(4.8)	7	(5.1)	14	(6.0)	2	(1.8)	6	(3.4)
o	6th sternebra: Incomplete ossification	Variant	14	(9.2)	22	(16.2)	34	(19.3)	25	(14.9)	19	(11.7)
	Pelvic girdle											
39	Entire: Asymmetric insertion	Minor	1	(0.6)	0	(0.0)	1	(0.5)	1	(0.5)	1	(0.4)
40	Pubis- Uni- or bilateral: Not ossified	Minor	0	(0.0)	1	(0.6)	2	(1.2)	1	(0.7)	2	(1.0)
41	Pubis- Uni- or bilateral: Incomplete ossification	Minor	1	(0.5)	3	(2.0)	11	(7.1)	1	(0.6)	16	(8.5)
	Forelimb											
p	Epiphyses: Not ossified	Variant	31	(18.5)	33	(22.8)	54	(28.8)	31	(20.9)	44	(26.4)
q	Proximal or distal epiphyses of humerus only: Not ossified	Variant	35	(21.6)	34	(24.3)	45	(26.4)	33	(19.5)	37	(23.0)

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Study number : SPD/021/R - Oral (Gavage) Rabbit Developmental Toxicity Study

TABLE 11 (continued)

Skeletal examination of foetuses : group mean data
Number of foetuses affected (group mean percent)

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4		Group 5	
Total number of foetuses examined			152		133		180		153		158	
Total number of litters examined			16		16		18		17		18	
Forelimb (continued)												
42	One or more metacarpal: Not ossified	Minor	1	(0.5)	2	(1.1)	4	(2.9)	0	(0.0)	5	(2.2)
43	One or more phalange: Not ossified	Minor	0	(0.0)	3	(1.7)	2	(1.2)	1	(0.6)	2	(1.2)
r	One or more phalange: Incomplete ossification	Variant	13	(8.1)	49	(35.6)	37	(21.8)	30	(22.5)	48	(28.9)
Hindlimb												
s	Epiphyses: Not ossified	Variant	80	(49.2)	63	(43.5)	94	(50.6)	81	(49.2)	88	(55.2)
t	Proximal epiphyses of tibia or distal epiphyses of femur only: Not ossified	Variant	46	(32.8)	52	(40.6)	56	(32.4)	50	(35.6)	45	(28.8)
44	Astragalus- Uni- or bilateral: Not ossified	Minor	0	(0.0)	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)
45	One or more phalange: Not ossified	Minor	0	(0.0)	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)
u	One or more phalange: Incomplete ossification	Variant	1	(0.5)	8	(5.0)	5	(3.7)	6	(3.9)	10	(6.1)

TABLE 2: Mean (± S.D., n = 3) plasma concentrations (ng/ml) and some pharmacokinetic parameters of **galantamine** after single (day 6 of pregnancy) and repeated (day 18 of pregnancy) administration of an aqueous solution of galantamine hydrobromide (R113675) at 5, 15, 30 and 40 mg/kg/day in an oral (gavage) rabbit developmental toxicity dose ranging study

Dose	4 8 mg/kg		12 16 mg/kg		24 30 mg/kg		32 40 mg/kg	
Time (h)	single	repeated	single	repeated	single	repeated	single	repeated
0	≤ 8.0	-1)	≤ 8.0	-1)	≤ 8.0	≤ 8.0	≤ 8.0	-1)
1	≤ 8.0	≤ 8.0	199 ± 106	388 ± 164	560 ± 481	1007 ± 90	198 ± 206	1001 ± 186
2	≤ 8.0	≤ 8.0	83.9 ± 52.0	163 ± 78	200 ± 109	476 ± 164	169 ± 78	746 ± 111
4	≤ 8.0	≤ 8.0	17.1 ± 11.5	43.3[2]	23.1 ± 5.3	127 ± 83	88.6 ± 81.6	342 ± 227
8	≤ 8.0	≤ 8.0	≤ 8.0	≤ 8.0	≤ 8.0	≤ 8.0[3]	≤ 8.0[3]	12.1[3]
24	≤ 8.0[3]	≤ 8.0	≤ 8.0	≤ 8.0	≤ 8.0	≤ 8.0	≤ 8.0	≤ 8.0
C_{max} (ng/ml)	-4)	-4)	199 ±106	388 ±164	560 ±481	1007 ± 90	241 ± 177	1001 ±186
T_{max} (h)	-4)	-4)	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1.7 ± 0.6	1 ± 0
$t_{1/2}$ (h)	-4)	-4)	1.1 ± 0.4	0.9[2]	0.7 ± 0.1	1.0 ± 0.1	2.3 ± 2.1	1.1 ± 0.3
$AUC_{0\text{-}24\ h}$ (ng.h/ml)	-4)	-4)	369 ±189	885[2]	905 ±651	2088 ±505	839 ± 248	3234 ±946

1) No sample collected.
2) n = 2.
3) Median.
4) Parameter not calculated or determined.

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TABLE 3: Mean (± S.D., n = 3) plasma concentrations (ng/ml) of **norgalantamine** after single (day 6 of pregnancy) and repeated (day 18 of pregnancy) administration of an aqueous solution of galantamine hydrobromide (R113675) at 5, 15, 30 and 40 mg/kg/day in an oral (gavage) rabbit developmental toxicity dose ranging study (Study Number:

Dose	4 ~~5~~ mg/kg		12 ~~15~~ mg/kg		24 ~~30~~ mg/kg		32 ~~40~~ mg/kg	
Time (h) (post-dosing)	single	repeated	single	repeated	single	repeated	single	repeated
0	≤ 20	-1)	≤ 20	-1)	≤ 20	-1)	≤ 20	-1)
1	≤ 20	≤ 20	27 ± 2	53 ± 22	-2)	-2)	≤ 20	-2)
2	≤ 20	≤ 20	≤ 20	24[3])	-2)	-2)	≤ 20	-2)
4	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20	-2)	-2)	-2)
8	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20
24	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20	-2)	-2)

1) No sample collected.
2) Interfering peaks.
3) Median.

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GENOTOXICITY

1) Ames Tests

a) Performed by

Batch # 20893

Strains: Salmonella TA 1535, TA 1537, TA 1538, TA 98, and TA 100

method used. S9 derived from livers of
- treated rats. HD = 5000 ug/plate (no bacterial toxicity seen). Two complete assays were done; an additional assay was done in selected strains due to equivocal effects in the second assay. Plating was done in triplicate for each assay

Galantamine was negative overall.

b) Performed by:

Batch # not stated

Strains: Salmonella TA 1535, TA 1537, TA 1538, TA 98, and TA 100

method used. S9 derived from livers of
treated rats. HD = 4000 ug/plate (limited by solubility; no bacterial toxicity seen). A single assay was performed; plating was in triplicate.

Galantamine was negative.

c) Performed by:

Batch # 23266

Strains: E. coli WP2 uvr A and WP2 uvr A pkM 101

' method used. S9 derived from livers of
treared rats. HD = 5000 ug/ plate (no bacterial toxicity seen). Two complete assays were done; an additional assay was done in one of the strains (see below). Plating was in triplicate for each assay.

Results are shown in the attached tables. In "Experiment 1", statistically significant increases were said to have been seen, at the 2 highest drug concentrations, with WP2 uvr A in the presence of metabolic activation. As shown in the table, the increases were only 1.6x the negative control; on the other hand the positive control was only 2.2x the negative control. Also shown in this table is that the positive control for the WP2 uvr A pkM 101 strain in the presence of activation was only 1.6 x the negative control; drug groups showed sporadic slight non dose - related increases of up to 1.3 x.

In "Experiment 2", the increase in revertants of WP 2 uvr A in the presence of activation was not repeated, although again, the positive control response was only 2.5 x negative control. The results with WP2 uvr A pkM 101 in the presence of activation were similar to those in Experiment 1, i.e. slight non D -R increases in the drug groups (maximum 1.4 x) with a relatively weak positive control response (2.1 x).

In "Experiment 3," the increase in revertants of WP2 uvr A in the presence of activation was again not repeated, although again the positive control response was not strong (2.6 x). (The other strain was not re-tested).

Overall, there is no evidence that galantamine was positive in these assays, although their sensitivity may be questioned.

2) Mouse lymphoma assay (TK locus)

Performed by

Batch # 23266

The methods were only briefly described. Drug treatment time was 3 hours, followed by a period of unstated duration when cells were examined "daily." Incubation period after plating was 10-12 days. S9 derived from

treated rats. Two assays were done. Drug concentrations were 250, 500, 2500, and 5000 ug/ml (which apparently showed little or no cytotoxicity in a rangefinding study).

Results were only minimally provided; e.g. degree of survival in the main study was not given, nor were individual culture data shown (and it is not clear how many replicates were used). According to the text, in the first assay an increased mutation frequency was seen only at the 500 ug/ml concentration in the presence of activation only; as this was not seen in the second assay, galantamine was considered to be negative overall.

3) In vitro cytogenetics in CHO cells

Performed by

Batch # 23266

Treatment time in the presence of S9 was 3 hours; in the absence of S9 treatment was continued until harvesting. Harvesting was after 1.5 cell cycle times (presumably 19.4 hr. based on a stated cycle time of 12.9 hr.); a second experiment was done in which an additional harvest at 24 hr. after the first harvest was done. In most cases 200 cells per drug concentration were scored (although it is not clear if these cells came from 1 or 2 cultures per group). The S9 was derived from . treated rats.

The drug concentrations used were based on a rangefinding study in which it was said that no toxicity was seen in the presence of S9 at drug concentrations up to 5000 ug/ml (data not shown), and the following toxicity was seen in the absence of S9:

Dose (ug/ml)	Mitotic Index (% of control)
50	97
100	50
500	36
1000	23
5000	0

(Note that in the main study, in contrast to the above statement, some reduction of mitotic index was seen in the presence of S9 at 5000 ug/ml, and toxicity in the absence of S9 appeared to be less than the above; see attached tables).

Chromosome aberration results are shown in the attached tables. (Statistical analyses shown in separate tables). Sporadic increases (including those where gaps were excluded) were seen in the drug groups which occasionally reached or approached statistical significance. (Note that the table of statistical results has separate analyses "By Totals" or "By Frequency" ; these terms are not defined [nor is mention made of this dual analysis in the methods section]; from a statement made in the text the "By Totals" analysis was based on number of cells with aberrations). There did not seem to be a consistent increase in any one type of aberration. (Note that when looking at individual types in the table, the number of cells scored in the negative control was 2x greater than that in the drug groups). The increases were not clearly dose related, were much lower than those in the positive controls (although no positive controls were used for the 2nd harvest in the 2nd experiment), and were said to be within historical control limits (although the latter were not provided).

4) Mouse micronucleus test

Performed by

Batch # 23266

15/sex were given a single gavage dose of 0, 6.4, 10, or 16 mg/kg. Five/sex/group were scheduled to be sacrificed 24, 48, and 72 hr. post-dose; this number was reduced in some cases due to deaths which ocurred in several animals immediately post dose. (At 48 hours, N=4 in HD M and N=3 in HD F; at 72 hours, N=3 in HD M and 0 in HD F). A minimum of 1000 PCE per animal were scored.

"Severe toxicity" was seen in surviving HD. There were no drug effects on micronuclei. The positive control (tested at 24 hr. point only) produced a large effect.

5) Assays with norgalantamine

An Ames test, an in vitro cytogenetic study in CHO cells, and a mouse micronucleus assay were performed. These were done by the same lab, and used

similar methods and drug concentrations (with the exception that HD in the mouse micronucleus assay was 40 mg/kg), as the above studies 1a, 1c, 3, and 4 done with the parent compound. Norgalantamine was negative in these assays.

APPEARS THIS WAY
ON ORIGINAL

Ames (E. coli)

6.2 Mutation Experiment 1

The responses of the test strains to the negative control, Galanthamine Hydrobromide and the positive controls are given as individual plate counts in Appendix 1.

All solvent controls gave counts of spontaneous revertants within expected ranges.

All positive controls gave counts of induced revertants within expected ranges.

6.2.1 Mean Results

MEAN NUMBER OF REVERTANTS PER PLATE EXPERIMENT 1								
		Concentration of test substance (μg/plate)						
Strain	% S-9	0	8	40	200	1000	5000	PC
WP2uvrA	0	57.0	43.3	47.0	60.3	49.7	48.7	498.7
WP2uvrApKM101	0	220.7	245.3	254.3	240.7	234.3	203.3	1229.7
WP2uvrA	10	57.7	63.0	57.7	63.0	93.0	92.0	128.3
WP2uvrApKM101	10	238.0	251.3	290.7	309.3	266.0	249.0	373.7

PC = Positive Control

6.2.2 Statistical Analysis

Dunnett's test was carried out on the data from experiment 1.

t-statistic from Dunnett's test in Experiment 1								
		Concentration of test substance (μg/plate)					Degrees of freedom	
Strain	% S-9	8	40	200	1000	5000	N	D
WP2uvrA	0	-2.32	-1.64	0.53	-1.20	-1.33	5	12
WP2uvrApKM101	0	1.52	2.03	1.25	0.83	-1.12	5	12
WP2uvrA	10	0.86	0.03	0.78	4.97*	4.85*	5	12
WP2uvrApKM101	10	0.35	1.64	2.18	0.89	0.34	5	12

* = $p < 0.05$

Ames (E. coli)

6.3 Mutation Experiment 2

The responses of the test strains to the negative control, Galanthamine Hydrobromide and the positive controls are given as individual plate counts in Appendix 2.

All solvent controls gave counts of spontaneous revertants within expected ranges.

All positive controls gave counts of induced revertants within expected ranges.

6.3.1 Mean Results

MEAN NUMBER OF REVERTANTS PER PLATE EXPERIMENT 2								
		Concentration of test substance (μg/plate)						
Strain	% S-9	0	8	40	200	1000	5000	PC
WP2uvrA	0	20.3	19.0	22.0	17.7	20.0	18.3	587.3
WP2uvrApKM101	0	259.7	282.7	282.0	285.3	275.7	197.0	750.3
WP2uvrA	10	16.0	20.3	18.3	17.3	17.0	22.3	39.7
WP2uvrApKM101	10	196.7	243.0	276.7	272.3	282.7	253.3	417.3

PC = Positive Control

6.3.2 Statistical Analysis

Dunnett's test was carried out on the data from experiment 2.

t-statistic from Dunnett's test in Experiment 2								
		Concentration of test substance (μg/plate)					Degrees of freedom	
Strain	% S-9	8	40	200	1000	5000	N	D
WP2uvrA	0	-0.25	0.41	-0.59	-0.00	-0.41	5	12
WP2uvrApKM101	0	1.06	1.04	1.20	0.75	-3.22	5	12
WP2uvrA	10	1.16	0.63	0.32	0.20	1.62	5	12
WP2uvrApKM101	10	1.49	2.46	2.33	2.59	1.81	5	12

Ames (E. coli)

6.4 Mutation Experiment 3

The responses of the test strain to the negative control, Galanthamine Hydrobromide and the positive controls are given as individual plate counts in Appendix 3.

The solvent control gave counts of spontaneous revertants within expected ranges.

Both positive controls gave counts of induced revertants within expected ranges.

6.4.1 Mean Results

MEAN NUMBER OF REVERTANTS PER PLATE EXPERIMENT 3								
		Concentration of test substance (μg/plate)						
Strain	% S-9	0	8	40	200	1000	5000	PC
WP2uvrA	0	16.3	18.7	18.3	16.3	19.7	17.3	480.0
WP2uvrA	10	19.0	18.7	22.0	17.0	20.7	16.3	50.0

PC = Positive Control

6.4.2 Statistical Analysis

Dunnett's test was carried out on the data from experiment 3.

t-statistic from Dunnett's test in Experiment 3								
		Concentration of test substance (μg/plate)					Degrees of freedom	
Strain	% S-9	8	40	200	1000	5000	N	D
WP2uvrA	0	0.53	0.43	-0.05	0.79	0.17	5	12
WP2uvrA	10	-0.08	0.52	-0.44	0.29	-0.69	5	12

Experiment 1

Table 2

CHO

Dose	Total	Aberrant	Mitotic		Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations	
(µg/ml)	cells	cells	Index	Gaps	deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps
Without Metabolic Activation 1st harvest														
100	200	18	4.04	8	3	0	6	5	0	12	0	26	9	5.5
500	200	16	3.35	3	3	0	4	6	0	5	0	22	8	6.5
1000	200	23	2.5	8	10	0	3	5	1	13	0	25	11.5	8
Control	400	30	6.48	13	4	0	4	14	0	16	0	45	7.5	4.5
MMC	50	38		11	21	32	1	0	10	1	0	4	76	70
With Metabolic Activation 1st harvest														
500	200	9	2.45	2	2	1	1	3	0	8	0	23	4.5	3.5
1000	200	18	2.5	11	3	0	3	3	0	15	0	28	9	4.5
5000	200	14	1.8	5	2	2	1	5	0	7	0	12	7	4.5
Control	400	20	2.85	6	4	2	5	5	1	15	1	21	5	3.8
CPA	50	18		3	8	13	1	1	4	1	0	1	36	34

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Experiment 2

CHO

Table 3

Dose	Total	Aberrant	Mitotic		Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations	
(µg/ml)	cells	cells	Index	Gaps	deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps
Without metabolic activation 1st harvest														
100	200	16	4.75	7	4	0	6	3	0	20	0	16	8	5
500	200	9	10.15	2	3	2	2	1	0	16	0	16	4.5	3.5
1000	200	7	3.60	1	3	0	4	0	0	12	0	10	3.5	3.5
Control	400	24	5.90	13	8	0	4	0	0	19	0	29	6	2.8
MMC	50	25		2	17	10	0	0	5	7	0	1	50	46
With metabolic activation 1st harvest														
500	200	20	5.20	12	0	0	4	7	0	19	0	20	10	5
2500	200	16	6.40	6	2	0	8	5	0	8	0	26	8	5.5
5000	161	15	4.25	5	3	1	4	3	0	8	0	16	9.3	6.2
Control	400	30	6.63	11	5	0	6	9	0	22	0	27	7.5	4.8
CPA	50	20		1	14	6	0	1	5	12	0	7	40	38
Without metabolic activation 2nd harvest														
100	200	11	10.95	2	5	0	3	1	0	12	0	2	5.5	4.5
500	200	8	10.20	2	5	0	1	2	0	14	0	5	4	3
1000	200	13	8.75	2	5	0	15	2	0	14	0	8	6.5	5.5
Control	400	15	6.01	1	7	0	14	2	3	16	0	18	3.8	3.5
With metabolic activation 2nd harvest														
500	200	22	13.45	13	5	1	2	3	0	19	0	4	11	4.5
2500	200	8	15.25	6	7	0	0	0	0	15	0	1	4	1
5000	200	9	11.70	4	3	0	1	1	0	16	0	4	4.5	2.5
Control	400	33	12.58	22	9	0	6	3	0	25	0	10	8.3	3.3

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Summary of Statistical Analysis

All experimental points were compared statistically by the Fisher's exact test. with the relevant solvent controls.

CHO

EXPERIMENT 1

Table 4

					Fisher's probability (mid-Z)			
Dose	By Totals				By Frequency			
µg/ml	With gaps		Without gaps		With gaps		Without gaps	
Without Metabolic Activation 1st harvest								
100	0.31	NS	0.36	NS	0.23	NS	0.29	NS
500	0.47	NS	0.20	NS	1.00	NS	0.37	NS
1000	(0.07)	NS	(0.06)	NS	0.07	NS	(0.08)	NS
MMC	0.00	<***	0.00	<***	0.00	A>N	0.00	A>N
With Metabolic Activation 1st harvest								
500	1.00	NS	1.00	NS	1.00	NS	1.00	NS
1000	0.05	*	0.40	NS	(0.03)	*	0.46	NS
5000	0.21	NS	0.40	NS	0.22	NS	0.35	NS
CPA	0.00	<***	0.00	<***	0.00	<***	0.00	<***

NS = Not significant
* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$
<*** = $p < 0.00005$
A>N = Number of aberrations is greater than the number of cells scored

CHO

EXPERIMENT 2

Table 5

Fisher's probability (mid-Z)								
Dose	By Totals				By Frequency			
µg/ml	With gaps		Without gaps		With gaps		Without gaps	
Without Metabolic Activation 1st harvest								
100	0.22	NS	0.12	NS	0.07	NS	0.04	*
500	1	NS	0.39	NS	1	NS	0.34	NS
1000	1	NS	0.39	NS	1	NS	0.46	NS
MMC	0	<***	0	<***	0	<***	0	<***
With Metabolic Activation 1st harvest								
500	0.19	NS	0.52	NS	0.09	NS	0.47	NS
2500	0.47	NS	0.41	NS	0.16	NS	0.15	NS
5000	0.29	NS	0.3	NS	0.25	NS	0.25	NS
CPA	0	<***	0	<***	0	<***	0	<***
Without Metabolic Activation 2nd harvest								
100	0.22	NS	0.35	NS	1	NS	1	NS
500	0.52	NS	1	NS	1	NS	1	NS
1000	0.1	NS	0.17	NS	0.01	**	0.02	*
With Metabolic Activation 2nd harvest								
500	0.17	NS	0.29	NS	0.27	NS	0.36	NS
2500	1	NS	1	NS	1	NS	1	NS
5000	1	NS	1	NS	1	NS	1	NS

NS = Not significant
* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$
<*** = $p < 0.00005$

SUMMARY

A) Pharmacodynamics

The primary mechanism of action of galantamine (G) in Alzheimer's disease is believed to be reversible, competitive inhibition of acetylcholinesterase (AChE). The results of pharamcodynamic studies will not be noted here due to time constraints; however , the following findings concerning (1) a possible nicotinic agonist effect of G and (2) anticholinesterase activity of G metabolites are noted due to their mention in the proposed labeling:

(1) Several published articles (with overlapping authors) were included which examined a potential agonist effect of G at nicotinic receptors in vitro. It was concluded that G (as well as physostigmine) induces single channel activity similar to that induced by acetylcholine and classical nicotinic agonists in a variety of cell culture preparations. Based on the use of classical nicotinic antagonists and other compounds, it was concluded that G acts at a different site from that of acetylcholine (although in one preparation, nicotinic antagonists did block the effect). Potentiation by G of the single channel activity induced by acetylcholine was shown. Other data indirectly suggested that these effects of G and physostigmine were unrelated to cholinesterase inhibition. The concentrations at which this agonist effect of G occurs is not clear, and little D-R data were shown; for example some studies concluded agonist activity was seen at 1 uM, with higher concentrations producing desensitization or antagonism, yet other studies showed activity at 50 uM (the only concentration used). The biphasic D-R curve (i.e. desensitization at higher concentrations) was given as a possible reason why G, although able to induce single channel currents, could not induce whole cell responses.

In studies conducted by the sponsor, G did not display significant nicotinic agonist or antagonist properties in human neuroblastoma cells. Although standard receptor binding studies were performed with G, nicotinic binding was apparently not studied.

(2) A study was performed to determine the degree of inhibition of AChE in human RBC and butyrylcholinesterase (BChE) in human plasma by G metabolites in vitro. Ki values (uM) were as follows:

	AChE	BChE
*Galantamine	0.84	2.59
Norgalantamine	0.83	4.05
O-desmethylnorgalantamine	0.53	2.11
O-desmethylgalantamine	0.19	4.60

Galantamine N-oxide, epigalantamine, and galantaminone were inactive at the highest concentration tested (10 uM).

* Note that the values for galantamine were obtained in a separate study ("under exactly the same conditions").

B) ADME/PK

The sponsor's summary is attached. The following discussion addresses the exposure to parent compound achieved in the animal carcinogenicity and reproduction studies compared with that in humans receiving the maximum recommended dose. This is followed by a discussion of the metabolic profile of galantamine, specifically regarding how this might affect conclusions regarding comparisons of exposure to the parent compound.

The following are calculations of the animal/human plasma AUC ratios for parent drug using data from selected animal studies. (The actual animal data are shown in association with the various studies earlier in this review). The units for AUC are ng.hr/ml, and are for $AUC_{0\text{-}24\ hours}$ unless otherwise indicated. The AUC value used for humans at the maximum recommended daily dose (16 mg b.i.d.) is 1840 (as given in volume 1.8, p. 255). All doses are in mg/kg. **(NOTE ADDED IN PROOF 7/17/00: It has been decided that the MRHD will be 12 mg b.i.d.; based on a human AUC of 1340 [vol. 1.8, p. 255], the following ratios should be multiplied by 1.37).**

1) Mouse 2 year carcinogenicity study.

	AUC Ratio	
Dose	M	F
2.5	0.4	0.2
5	0.6	0.4
10	1.5	0.8

However, values obtained at the end of a 3 month rangefinding study at 10 mg/kg (lower doses not used) were about half those obtained at this dose (at 6 months) in the carcinogenicity study. (AUCs in the transgenic mouse study were even lower than those in the 3 month study, although the strain in the transgenic study was different). The following ratios were calculated using the average of the AUC values determined in the 3 month and 2 year studies. (Doses of 2.5 and 5 mg/kg were not used in the 3 month study; for the calculations below it is assumed that the values were lower to the same relative degree as that seen at 10 mg/kg):

	AUC Ratio	
Dose	M	F
2.5	0.3	0.2
5	0.5	0.3
10	1.2	0.6

2) Mouse transgenic carcinogenicity study

	AUC Ratio	
Dose	M	F
2.5	0.1	0.1
5	0.3	0.2
10	0.6	0.4
20	2.3*	1.0

*Carcinogenicity not adequately evaluated at this dose in M due to excessive mortality.

3) Rat 2 year carcinogenicity study

(Calculations based on the average of the AUC values obtained on days 182 and 398)

	AUC Ratio	
Dose	M	F
2.5	0.5	1.1
10	2.6	4.1
30	8.6	13.9

4) Rat rangefinding reproduction study

(M treated from 2 weeks pre-mating for 5 weeks. F treated from 2 weeks pre-mating through day 7 of pregnancy. Plasma levels measured on first and last days of treatment. AUC values were 0.5 - 8 hours, thus underestimating $AUC_{0\text{-}24\ hours}$.).

a) Results on first day of treatment:

AUC Ratio

Dose	M	F
2	0.2	0.3
8	0.9	1.3
16	1.2	1.5
32*	1.7	-

b) Results on last day of treatment:

AUC Ratio

Dose	M	F
2	0.2	0.5
8	1.1	2.0
16	2.2	3.4

*16 mg/kg was the highest dose used in the main rat reproduction studies.

5) Rabbit rangefinding reproduction study

(Rabbits treated days 6-18 of pregnancy; plasma levels measured on first and last day of treatment. Inter-individual variation was rather large, especially after the first dose).

a) Results on first day of treatment

Dose	AUC RATIO
4	*
12	0.2
24	0.5
32	0.5

b) Results on last day of treatment

Dose	AUC RATIO
4	*
12	0.5
24	1.1
32	1.8

(40 mg/kg was the highest dose in the main study).

* levels below limit of quantification

Galantamine was metabolized to various degrees depending on species. (Figure showing proposed metabolic pathways is attached). In Wistar rats after 2.5 mg/kg p.o. (base), 16% and 33% of the dose was excreted in urine as unchanged drug in M and F, resp. ; in another study in males this value was 13% after a dose of 10 mg/kg p.o. (base). In humans, these values for poor metabolizers and extensive metabolizers (N=2 males each; each pair was pooled) were 39% and 24%, respectively. (I am not aware of analogous data for mice or rabbits). In studies measuring the plasma AUC for total label and unchanged galantamine after acute oral administration of labeled galantamine, values obtained for the ratio AUC galantamine/AUC total label were as follows: Wistar rats : 0.50 in females after 2.5 mg/kg (base), 0.23 in males after 2.5 mg/kg (base), and 0.06 after 10 mg/kg (base) (each of the above values obtained in a separate study); female "albino" rabbits : $\leq$ 0.01 after 10 mg/kg (base); male humans : 0.32 (range 0.19 - 0.45 over the 4 subjects studied) after 4 mg base. In humans (2 M and 2 F) receiving 10 mg (hydrobromide salt) t.i.d. for 12 weeks, unchanged drug was the most abundant compound (of the various compounds measured) in plasma; the next most abundant was the conjugate of O-desmethyl galantamine (~1/2 - same as parent in 3/4 subjects; not detectable in the fourth), followed by the N oxide metabolite (~ 1/5-2/3 parent) and conjugated galantamine (similar amount to N oxide), and lesser amounts of conjugated O-desmethyl norgalantamine > norgalantamine > conjugated norgalantamine. In rats receiving 40 mg/kg (base) p.o. for up to 1 month, unchanged galantamine was also the most abundant of the compounds measured in plasma, and the above - mentioned human metabolites were also seen although in somewhat different ratios to the parent compound, e.g. the conjugate of O-desmethylgalantamine and the N-oxide metabolite were relatively lower compared to humans (former metabolite ~ 1/4 parent in male rats and ~1/7 parent in female rats; latter metabolite ~ 1/6 parent in male rats and ~1/20 parent in female rats). In 2 other studies in Wistar rats, using single p.o. doses of 2.5 or 10 mg/kg (base), levels of these 2 metabolites, relative to parent drug, were even lower than the above. In a study in female "albino rabbits" given a single p.o. dose of 10 mg/kg

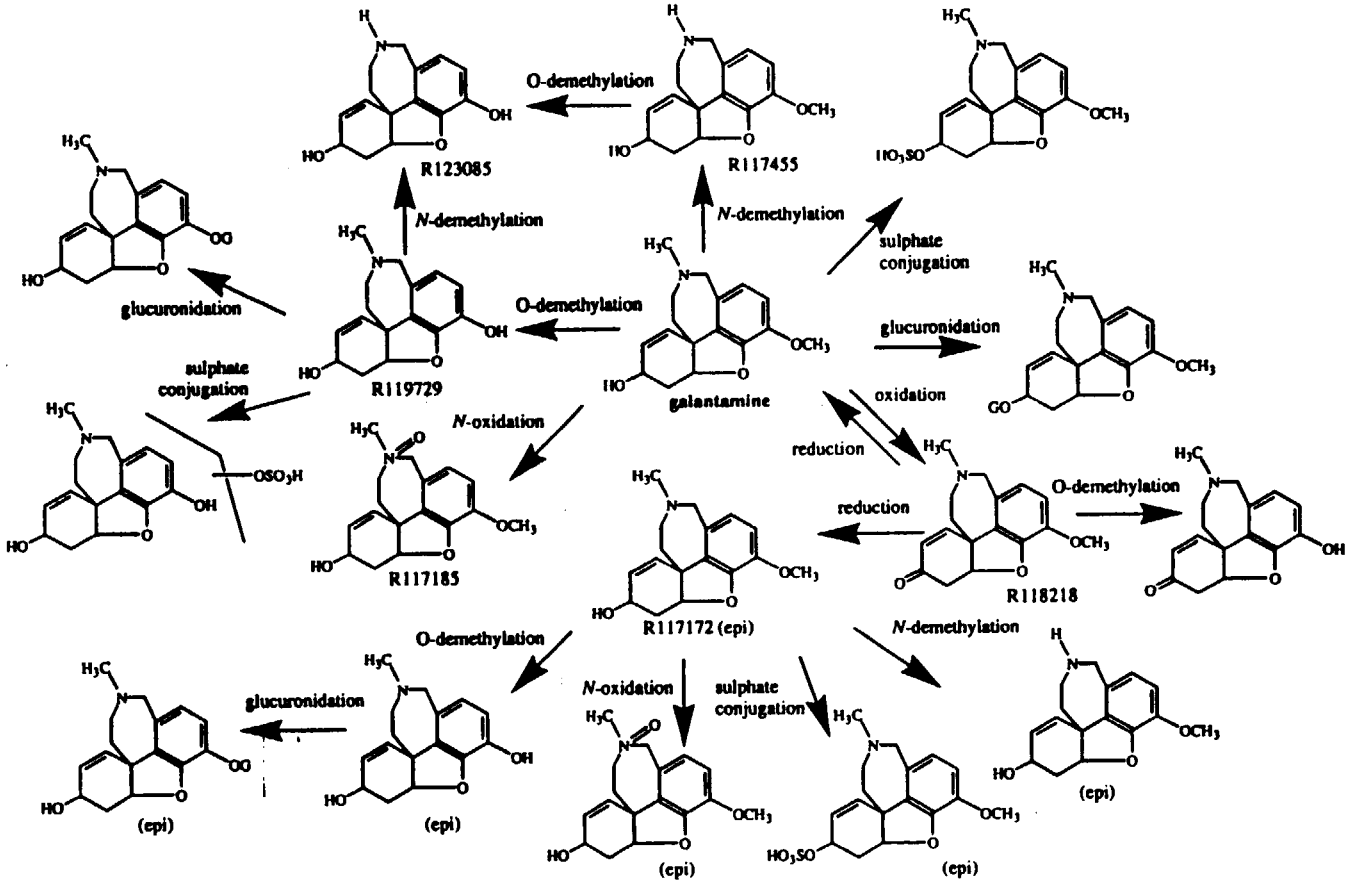
(base) , only very small amounts of unchanged or conjugated galantamine were seen (unlike the case in humans and rats, above). Of the compounds measured , only O-desmethylgalantamine glucuronide was present in appreciable quantities. A small amount of norgalantamine was seen (slightly greater than parent compound); no conjugated norgalantamine, conjugated O-desmethyl norgalantamine, or free O-desmethylgalantamine or norgalantamine were detected; the N-oxide metabolite was apparently not assayed for. No data on plasma levels of individual metabolites in mice were presented, aside from a TK study in which levels of norgalantamine were measured (AUC 1/5 and 1/2 that of parent in M and F, respectively).

Note that much of the above - discussed data are not very well established , e.g. sample sizes were relatively small , and plasma levels of metabolites were measured at one or a few time points, i.e. AUC comparisons were not available.

Plasma protein binding is not a complicating factor in comparing plasma levels across species; it was less than 30% across all species studied (humans, rats, mice, rabbits, dogs).

In sum, it appears that comparison of human exposure (i.e. to plasma levels of parent drug) with that of the Wistar rat is reasonable, although such a comparison will underestimate relative human exposure to the O-desmethylgalantamine conjugate and N oxide metabolites. (Note that the rat carcinogenicity study was done in Wistar rats, but the reproduction studies were done in Sprague Dawley rats). On the other hand, comparison of humans with rabbits is problematic; the latter had little or no unchanged drug in plasma and the N-oxide was not assayed for. Relevant information in mouse is not available, except that exposure to norgalantamine was relatively low (as is the case in humans).

Figure 7-1: Major metabolic pathways of galantamine after oral administration in animals and in man, as derived from the identification of the metabolites in urine, faeces and plasma.



C) Rat 1 Year Toxicity/2 Year Carcinogenicity Studies

Doses in the 1 and 2 year studies were as follows:

1 year: 0, 1.6, 8, 16, and 32 mg/kg, by gavage

2 year: 0, 2.5, 10, and 40--> 20--> 30 mg/kg, by gavage.

The 1 year study included a 6 month interim sacrifice and (in C and HD only) a 4 week recovery period.

Note that the rat strains were different in the 2 studies (SD and Wistar in 1 and 2 year studies, respectively). Also, the batches of drug in the 2 studies were obtained from different sources.

Observed signs were seen at doses of 8 mg/kg and above and were attributed to cholinergic stimulation (e.g. tremors, lacrimation, salivation), although the finding of "behavioral change" at 8 mg/kg and above in the 1 year study was not defined. Excess mortality was seen at 32 mg/kg and above. (In the 2 year study the animals which died after the first 40 mg/kg dose were replaced; the dose was then reduced and no clearly drug-related mortality was seen thereafter).

Bodyweight gain was reduced in all groups except LD F in the 1 year study and LD M in the 2 year study. In the 2 year study, final weights were 92 and 80% of control in MD and HD M, respectively, and 97, 89, and 89% of control in LD, MD and HD F, respectively. Food consumption was usually decreased in groups with decreased bodyweight gain. (No clear effects at LD).

Several hematological and blood chemistry changes were seen; these will not be discussed here since in general they were slight in degree, did not increase over time, were not seen in both studies (in fact, opposite changes sometimes were seen between the 2 studies), and have no apparent toxicologic significance. (One partial exception to the above characterization was a relatively large dose- and time-related increase in blood glucose in the 1 year study; mean values at HD reached 1.7 x control. However, no effect was seen in the 2 year study).

The only tumor types which appeared to be increased by drug in the 2 year (carcinogenicity) study were uterine adenocarcinomas at MD and HD (4/60, 3/60, 9/60, and 9/60 in C, LD, MD, and HD, respectively) and cervical sarcomas (3 in HD; none in the other groups; cervix was only examined in animals with gross lesions). The latency of detection of uterine adenocarcinomas was not clearly decreased in the drug groups. (Also, none were reported in the 1 year study). The incidence values of uterine adenocarcinomas was above the performing laboratory's historical control range; historical data were from 5 control groups in 3 studies; incidence values were 1/50, 2/50, 2/49, 3/50, and 4/50. (Historical control values for cervical sarcomas were 4 groups with

incidences of 0/50 and 1 with an incidence of 2/50; however it is not clear if these values can be compared with those in the present study since it is not clear if only animals with gross findings in cervix were examined as was done in the present study. [The importance of this problem depends on how likely cervical tumors are detected in the absence of gross findings]). The sponsor hypothesizes that the increase in uterine tumors was due to a hormonal mechanism, which will be discussed in the Evaluation section, below.

Other neoplastic findings in the 2 year study were a decreased incidence of pituitary adenomas in all groups but LD F (a trend in same direction also seen in 1 year study), and a decreased incidence of mammary adenocarcinomas and fibroadenomas in HD F.

Several non-neoplastic effects were seen; the most prominent were:

1. Changes in female genital tract (ovary, uterus, vagina) said to be reflective of increased cyclic activity, primarily at HD (but sometimes extending to lower doses), in the 2 year study.

2. Decreased mammary gland activity at HD F in the 2 year study.

3. Increased incidence of foamy macrophage accumulation in lung at the higher doses in the 1 year study. The absolute incidence value was relatively low, i.e. 23% at HD (vs 2% in controls). Reversibility was equivocal. (In the 2 year study, there was an equivocal increase in incidence of macrophages or "foamy cells" in HD F).

4. Increased incidence of hypertrophy of mandibular salivary gland at MD and above in the 1 year study. Most rats were affected at the 2 highest doses. It was not seen after the recovery period. There were no effects on mandibular salivary gland in the 2 year study; a slight increase in "focally large basophilic acini" in parotid salivary gland was seen in HD F.

5. Decreased incidences of pathologic changes in various organs were seen in the 2 year study as listed earlier. A decreased incidence of chronic renal disease was seen in both studies.

D) Mouse Carcinogenicity Studies

A 2 year carcinogenicity and a P53 transgenic study were performed.

Doses in the 2 year study were 2.5, 5, and 10 mg/kg by gavage. Slight sedation was seen at HD. (In a 3 month rangefinding study, 20mg/kg caused sedation and tremors). There was no clear overall drug effect on mortality in males; in females 3 of

the first 10 treated HD died on day 1 (after which the dose was decreased to 5 mg/kg and then gradually increased back to 10 mg/kg); overall mortality in females was slightly less than controls in all drug groups but not D-R. (In the rangefinding study, 20 mg/kg and 40 mg/kg caused 50% and 100% lethality, respectively). Bodyweight gain was decreased at all doses, D-R (but generally not statistically significant in LD F). Weights near the end of the study in males at LD, MD and HD were 97%, 90% and 90% of control, respectively; corresponding values in F were 93%, 92% and 87%. Sporadic decreases in food consumption were seen but overall there was no clear drug effect. There were no pronounced effects in hematology and blood chemistry exams; slight effects included increases in Hb and occasionally RBC and Hct in HD M, sporadic decreases in WBC (total and individual cell types) at MD and HD, increased platelets at HD, decreased K in HD M, and decreased BUN in all M groups (not D-R). There were no clearly drug-related increases in neoplastic or non-neoplastic findings. There was a trend toward a slight decrease in chronic renal pathology at HD.

Doses in the transgenic P53 study were 2.5, 5, 10, and 20 mg/kg, by gavage. Sedation and tremors were seen at the 2 highest doses. Eight of 15 HD M died on the first day of dosing; after this scattered deaths were seen at the higher doses but in most cases were attributed to dosing accidents. (In a 1 month rangefinding study using the wild type strain, no deaths [out of 5/sex] were seen at the highest dose of 20 mg/kg. However, in a 6 month study in the wild type strain, 2 deaths [n=15/sex] were seen on the first day of dosing at 10 mg/kg [the only dose level used]). Bodyweight gain was decreased in all groups except only equivocally in LD F; weights near the end of the study in males at LD, MD, M-HD, and HD were 91%, 85%, 82%, and 85% of control , respectively; corresponding values in F were 96%, 93%, 89% and 89%. Sporadic decreases in food consumption were seen in all groups but LD M. There were no pronounced effects on hematology or blood chemistry; slight effects included decreased RBC, Hb, and Hct in all M groups (not D-R), decreased WBC (total and individual cell types) in all M groups except LD, increased K in all M groups, and decreased glucose in MD and HD (but not M-HD) M. There were no drug-related neoplastic or non-neoplastic effects except for a slight decrease in chronic inflammation of salivary gland in HD M.

(A 6 month study was also performed in the wild type strain, using a single dose level of 10 mg/kg. Results were generally comparable with the above, including the hematology [but not the clinical chemistry] findings and the decrease in chronic inflammatory cells in salivary glands in males [which was seen at 20mg/kg only in the former study]. No neoplasms were seen in any animals).

E) 1 Year Dog Toxicity Study

Beagles received 0, 1.6, 4, or 8 mg/kg/day, in capsules, for 1 year. Four/sex/group were sacrificed at 6 and 12 months, and 3/sex in controls and HD were kept for a 4 week recovery period.

Toxic signs included various nervous system effects, several likely due to acetylcholinesterase inhibition. Tremors, fasciculations, salivation, lacrimation, diarrhea, and hyperactivity were seen at all doses; emesis was seen at MD and HD only, and low incidences of ataxia , excessive panting, and hypoactivity were seen at HD only. A transient, slight increased weight loss/decreased weight gain was seen in some HD M near the beginning of the study.

There were no clear drug effects on ophthalmoscopic or EKG exams; one HD M had an isolated occurrence of ST depression which was not seen on subsequent days and was not associated with blood transaminase elevations or histologic changes in heart. Neither this nor isolated occurrences of S-A block in an HD F or 2^0 A-V block in an MD F can be concluded to be drug-related.

There were no clear drug effects on routine lab tests; a few slight/equivocal effects were seen as noted earlier.

There were two histological changes considered to be drug-related:

1) The incidence of focal or multifocal degeneration of the tunica muscularis of the urinary bladder was increased at HD. The incidence was increased in HD M at 6 but not at 12 months. The incidence was increased in HD F at both 6 and 12 months, but the severity was less at 12 months. The lesion was not seen in recovery dogs. Myodegeneration of urinary bladder was also seen in two 4-week dog studies, at a dose as low as 4 mg/kg. In one of these studies myodegeneration of stomach and duodenum was also seen. It was stated that these lesions "most likely represented a manifestation of the exaggerated parasympathomimetic pharmacologic effects of [the drug] on urinary bladder muscle...".

2) Increased incidence of pseudopregnancy associated with endometrial hyperplasia and a slight increase in the size and/or number of ovarian corpora lutea was seen at 12 months in 1/4 MD F and 2/4 HD F, and after the recovery period in 1/3 HD F. It was stated that the above changes "resulted from persistent stimulation by progesterone which is released by retained corpora lutea", likely due to "central cholinergic stimulation of gonadotropin secretion" secondary to the pharmacologic effects of the drug, but no data were presented on this point. (Note that an additional dog study was subsequently performed to assess the above findings. Two female beagles per sex were given daily doses equivalent to the LD and HD of the 1 year study and compared with 2 controls. All dogs were in the active luteal phase of their sexual cycle at the beginning of the study. Treatment was for 6 months. It was concluded that there were no drug-related histological effects on the female genital tract or on serum progesterone levels. [However, the following should be kept in mind when comparing the 1 year and 6 month studies: they were done at different facilities and Janssen, respectively), the drug batches used in the 2 studies were from different batches, with drug used in the 1 year study "somewhat less pure", no toxic signs aside from occasional slight emesis at HD were seen in the 6 month study despite the pronounced signs seen at

this dose in the 1 year study (it is not clear as to what time relative to dosing animals were observed in the former; it is possible that signs occurred but were not observed), drug-related histologic effects in ovary and uterus were not seen at the 6 month sacrifice in the 1 year study, and the N of the 6 month study (2/group) was extremely low (and incidence values in the 1 year study were ≤ 50%)]).

F) Reproduction Studies

The following studies were performed (mg/kg doses in parentheses):

1) Rat fertility/embryonic development (2, 8, 16)
2) Rat pre- and post- natal development (2, 8, 16)
3) Rabbit embryonic development (4, 12, 28, 40)

Doses for the rat studies were based on a rangefinding study in which 32 mg/kg caused severe signs and was not tolerated.

In the rat fertility/embryonic development study, M were treated from 60 days pre-mating through mating, and F were treated from day 14 pre-mating through day 17 of pregnancy. F were sacrificed on day 20; fetuses were examined for external, visceral, and skeletal abnormalities by standard techniques. Observed signs mainly consisted of tremors at MD and HD. Bodyweight gain and food consumption were slightly decreased at MD and HD. There were no drug effects on sperm number, motility, or morphology. There was a slight (13%) decrease in the number of pre-mating estrous cycles at HD, but there were no drug effects on mating or fertility. There were no drug effects on pre- or post- implantation loss. Fetal exams showed a slight increase in minor skeletal abnormalities, primarily "one or more sternebrae bilobed, bipartite, misshapen or misaligned" at MD and HD. The fetal incidences of this finding at MD (18%) and HD (16%) as well as the control incidence (7%) were above the historical range (0-5.1%), as was the incidence for total minor skeletal abnormalities at MD and HD (24%; historical range 0.7-13.4%; concurrent control 13%). The incidence of the skeletal variant vestigial 14th rib was slightly increased at HD; the incidence was within the sponsor's historical range.

In the rat pre- and post-natal development study, done at the same doses as the above, pregnant F were treated from day 6 of pregnancy through day 21 PP; 20/sex F_1 pups per group were retained for developmental and fertility assessment. As in the above study, tremors were the most prominent sign, although in the present study they only occurred at HD and were of relatively low incidence. As in the above study, Fo dam weight gain and food consumption were slightly decreased at MD and HD. (On day 20 of pregnancy, weights were 96% and 93% of control at MD and HD, resp,; on day 21 PP, 97% and 93%, resp.). There were no drug effects on duration of gestation, numbers of total and live pups born, pup sex ratio, pup anogenital distance, pup survival, various pre- and post-weaning F_1 developmental milestones (including E- maze learning), or on F_1 mating and fertility. The only drug related effect was a decrease in F_1 pup bodyweight at

MD and HD. On day 21 PP, mean pup weights were ~ 93% and 90% of control at MD and HD, resp. During the post-weaning period weights in MD and HD M remained below controls, whereas weights in MD and HD F returned to control level by week 7 PP.

Doses for the rabbit study were based on a rangefinding study in which pregnant rabbits were given up to 32 mg/kg/day and non- pregnant rabbits up to 48 mg/kg/day; the higher doses caused weight loss/decreased weight gain and decreased food consumption; tremors were seen in 2/4 at 48 mg/kg. In the main study, trembling was seen in 3 rabbits at M-HD (28 mg/kg) and aggressiveness, feet stamping, tremors, and reduced quantity/ liquid/ loose/ absent feces were seen at HD (40 mg/kg). Bodyweight loss followed by decreased gain, and decreased food consumption , were seen at M-HD and HD. (Mean weight on day 18 of pregnancy 95% and 93% of control, respectively). There were no clearly drug-related effects on pregnancy data or fetal exams.

G) Genotoxicity

Galantamine was tested in the following assays:

1) Ames tests (2 studies with Salmonella strains; 1 with E. coli strains)

2) Mouse lymphoma assay (TK locus)

3) In vitro cytogenetics in CHO cells

4) Mouse micronucleus assay

In general, these studies conformed to current testing guidelines. Galantamine was not clearly positive in any of the above, although the following caveats are noted:

1) In the Ames assay with E. coli strains, the positive control values in the presence of metabolic activation ranged from 1.6x - 2.6x those of the negative control; thus the sensitivity of the assay may be questioned. (Galantamine produced slight [less than those of positive controls] increases in revertants which were either not dose-related or not replicated in subsequent assays).

2) The methods and results of the mouse lymphoma assay were only briefly described; a more detailed report will be needed to support inclusion of this study in labeling.

3) In the CHO study, sporadic increases in chromosomal aberrations were seen in the galantamine groups. Although these are probably not real drug effects for reasons discussed earlier, part of the rationale for this conclusion is the sponsor's statement that the values were said to be within historical control

limits. The historical control data should be requested from the sponsor to verify this.

(A published study [Blagoeva et. al., Med. Biol. Inf. (4), 25-28, 1986], which reported the effects of "Nivalin" in a UDS assay in human lymphocytes, an Ames Test [Salmonella TA 97, 98, 100, and 102], and a mouse micronucleus test [s.c. dosing], was included. Although it was concluded that "Nivalin" was negative in these assays, (1) the composition of "Nivalin" was not given except for the statement that galantamine hydrobromide is its "active ingredient," (2) the methods and results were not presented in sufficient detail for independent review, (3) it is not clear to what degree the assays conformed to current guidelines, and (4) positive controls were not used in all cases).

A genotoxicity battery (Ames test, in vitro cytogenetics in CHO cells, mouse micronucleus assay) was performed with the metabolite norgalantamine, which was negative.

APPEARS THIS WAY
ON ORIGINAL

EVALUATION:

This NDA is approvable.

Animal toxicity studies performed with galantamine included 1 year studies in rats and dogs, 2 year studies in rats and mice, and a 6 month study in transgenic (P53) mice. Aside from the production of various "cholinergic" symptoms (typical of acetylcholinesterase inhibitors) at the higher doses, no pronounced toxicity was seen. Histopathologic effects, as described in detail earlier, included an increase in foamy macrophages in lung (rats only) and myodegeneration of urinary bladder, stomach, and duodenum (dogs only). Changes in female genital tract said to be reflective of increased cyclic activity were seen in rats. A decrease in chronic renal pathology was seen in both rats and mice. Decreases in incidence of mammary and pituitary tumors, and decreases in non-neoplastic findings in various other organs, were seen in rats.

The only tumor types which may have been increased by galantamine were uterine adenocarcinomas (MD and HD) and cervical sarcomas (HD) in rats. The former was not statistically significant (at alpha = .005 for common tumors); the latter was significant. (See Statistical Review by Dr. Koti). However, the incidence values for both tumors were above the historical ranges (although note that these ranges were based on only 5 control groups from 3 studies). (Evaluation of the effect on cervical tumors is complicated by the fact that cervix was only examined in rats with gross findings, which represented only a small fraction of treated rats; the difference between the discovered incidence and the true incidence of cervical tumors depends on the likelihood of presence of tumors in the absence of gross findings).

The sponsor proposes the following mechanism for production of uterine tumors by galantamine: Galantamine, by inhibiting acetylcholinesterase, elevates acetylcholine in the hypothalamus, which in turn results in increased dopamine release and consequent decrease in prolactin secretion. (References are cited concerning cholinergic involvement in prolactin secretion). Since prolactin increases with age and has luteotropic activity in rats, normally resulting in persistent corpora lutea and a state of "progesterone dominance" in aging rats, the galantamine-induced prolactin decrease results in luteolysis and new follicle development, and a state of "estrogen dominance" which results in prolonged endometrial stimulation and consequent endometrial tumorigenesis. (An additional, related mechanism is proposed whereby the decreased prolactin due to galantamine is secondary to galantamine- induced decreased bodyweight gain). Since prolactin is not luteotropic in humans, it is concluded that any effects of galantamine on prolactin in humans will not result in effects on the female genital tract similar to those seen in rats.

The relevance to humans not withstanding, it does not seem that the proposed mechanism has been well established for galantamine. In favor of the

mechanism, an apparent decrease in plasma prolactin was seen in HD females at termination in the carcinogenicity study. (Described as "apparent" since little information was given on methodology, e.g. it was not stated what time of day or how long after dosing samples were taken; also there appeared to be a large inter-animal variation [although the difference was said to be statistically significant]). Consistent with a decrease in prolactin in HD females were decreases in mammary gland activity and neoplasia, and ovarian changes reflecting increased cyclic activity, in this group. However, the link between reduced prolactin and the hypothesized state of "estrogen dominance" is less clear. Estrogen levels were not measured. Aside from the uterine tumors and the finding of increased granulocytic infiltration, there were no histological effects in uterus which might be indicative of increased stimulation (e.g. no drug effects on hyperplasia, metaplasia, or height of epithelium). It is also noted that whereas the decrease in prolactin (and the occurrence of most of the above - mentioned associated histological changes) was seen only in HD, uterine tumors were increased at both MD and HD; furthermore, even at HD, there was no correlation between prolactin level and the presence of uterine tumors in individual animals.

In sum, the carcinogenic potential of galantamine appears to be low. The only possibly drug-related effect occurred in 1 sex of 1 species only, and only in uterus and cervix. The increase in uterine adenocarcinomas was not statistically significant (at alpha = .005), and there was no clear evidence for a decreased latency of occurrence. On the other hand, the incidence of both the uterine adenocarcinomas and cervical sarcomas was above the historical control range. Also, as noted above, the incidence of cervical tumors may have been underestimated. It is recommended that the uterus/ cervix findings be included in the labeling (without mention of the proposed mechanism, which was not well-established as noted above). It is also recommended that the sponsor perform histopathology on cervix in all animals in all groups; results of this may result in modification of labeling in the future.

The only noteworthy effects in the reproduction studies were (1) a slight increase in minor skeletal abnormalities in a rat fertility/embryonic development study, and (2) a slight decrease in pup weights in a rat pre- and post-natal development study. Both of these effects occurred in conjunction with slight decreases in parental weight gain.

LABELING :

1) Description and Clinical Pharmacology (Pharmacodynamics) sections

In addition to its anticholinesterase action, the labeling states that galantamine is a "nicotine receptor modulator" (Description section) and that it "enhances the intrinsic action of acetylcholine on nicotinic receptors, probably through binding to an allosteric site of the receptor" (Pharmacodynamics section). As discussed in the Summary section of this review, there is some evidence for this additional action; however it is noted that it has only been shown in vitro (and only occurred in single channels, not in whole cells) and the relationship of effective concentrations to concentrations which inhibit acetylcholinesterase is not clear. Furthermore, it is not clear that other drugs marketed for Alzheimer's disease do not show this action (physostigmine was shown to share it); it would be misleading to list this action for galantamine only. Also, since the efficacy of galantamine in Alzheimer's disease has not been shown to differ from that of the marketed drugs, it would be misleading to imply an additional mechanism of action. It is thus recommended that this additional action not be included in the labeling.

It is also noted the proposed labeling differs from that of marketed products in some regards, e.g. the latter contain statements that there is no evidence that the drug alters the underlying dementing process, and that the effect of the drug might lessen as the disease progresses.

2) Carcinogenesis, Mutagenesis, and Impairment of Fertility section

The following is a suggested revision of this section. The rat carcinogenicity study is placed first (in accordance with precedent of putting positive results first), the finding of increased cervical sarcomas is added, and the statement regarding the proposed mechanism of production of these tumors is omitted (see Evaluation section of this review). The Mutagenesis section may have to be modified pending responses to question asked of the sponsor concerning some of these studies (see Summary section of review). Fertility results are added. Safety factors are re-calculated to reflect a human bodyweight of 60 kg. AUC ratios are not used for the fertility study since rat values were only based on levels measured through 8 hours post-dosing. **(NOTE ADDED IN PROOF 7/17/00: Safety factors in this and the Pregnancy sections reflect a MRHD of 12 mg. b.i.d.).**

Carcinogenesis:

Mutagenesis:

(Wording is adequate pending satisfactory response to our questions as noted earlier).

Fertility:

No impairment of fertility was seen in rats given up to 16 mg/kg/day (40x MRHD on a mg/kg basis or 7x on a mg/m^2 basis)

3) Pregnancy section

(The following is a suggested revision of this section. The finding that an increase in minor skeletal abnormalities was also seen at 8 mg/kg is added, as is the finding that the above were seen at doses causing some maternal toxicity. Safety factors are re-calculated to reflect a human bodyweight of 60 kg. AUC ratios are not used since rat values were only based on levels measured through 8 hours post-dosing. Pregnancy category is B since only minor effects were seen which are likely related to maternal toxicity).

4) Labor and Delivery section

Results of animal studies are not normally included in this section and should thus be removed.

RECOMMENDATIONS:

This NDA is approvable.

1) Recommendations concerning labeling are made above.

2) Questions concerning the genotoxicity studies (as discussed in the Summary section of this review) should be transmitted to the sponsor. (Note added in proof: questions were transmitted to sponsor by project manager 5/3/00).

3) In phase IV, the sponsor should perform histopathologic exams on cervix from all animals in the rat carcinogenicity study in order to help determine if a true drug effect on this organ was present. Labeling may need to be modified based on the results.

/S/

Barry N. Rosloff, Ph. D.

cc: NDA 21-169, original submission + division file
Rosloff
Fitzgerald 7/10/00
Fanari

Executive CAC
Date of Meeting: 6/6/2000

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Robert Osterberg, Ph.D., HFD-520, Alternate Member
Abigail Jacobs, Ph.D., HFD-540, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Team Leader
Barry Rosloff, Ph.D., HFD-120, Presenting Reviewer

Author of Minutes: Barry Rosloff

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

NDA # 21-169
Drug Name: galantamine (Reminyl)
Sponsor: Janssen

Mouse Carcinogenicity Studies

A two year bioassay in CD 1 mice (gavage doses of 2.5, 5, and 10 mg/kg) and a 6 month transgenic (P 53) study (gavage doses of 2.5, 5, 10, and 20 mg/kg) were performed. Both studies were adequate and no evidence of drug-induced carcinogenicity was seen.

Rat Carcinogenicity Study

A two year bioassay was performed in Wistar rats at gavage doses of 2.5, 10, and 30 mg/kg. The only remarkable findings were slight/equivocal increases in uterine adenocarcinomas at MD and HD and in cervical sarcomas at HD. The increase in uterine adenocarcinomas was not statistically significant but the incidence at MD and HD was above the sponsor's historical control range. There was no evidence for earlier onset of uterine tumors in the drug groups. The incidence of cervical sarcomas at HD was low but was statistically significant and slightly above the historical control range. The cervix may have been incompletely evaluated since only animals with gross findings were examined histologically.

Executive CAC Recommendations and Conclusions:

It was concluded that the studies performed did not indicate any carcinogenic potential of the drug. Dr. Rosloff indicated that the Division intends to request histological evaluation of cervix in all animals in the rat study as a phase IV commitment; if this evaluation indicates a drug effect it will be included in the labeling.

/S/ 6/13/00

Joseph F. Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
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/G. Fitzgerald, HFD-120
/B. Rosloff, HFD-120
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