V. Reproductive Toxicology

Note: In Dr. Barry Rosloff's excerpted reviews, doses refer to the HBr salt of citalopram, rather than the base.

A. Three Generation Rat Study (549/65 and 621/65) QA, but prior to GLP

Wistar rats - 0.8, 4.8, 16/32 (M/F) mg/kg p.o. gavage (batch VII)
12 M/gr (24 con); 24 F/gr (48 con) half killed on GD20 and half allowed to deliver

Dr Barry Rosloff's original review of the three generation study is excerpted below. Discussed here are several deficiencies that are of particular concern because the three generation study is the only study to incorporate a Segment I portion (other individual Segment II and III studies have been submitted). Firstly, corpora lutea were not counted, making it impossible to assess the effect of citalopram on preimplantation events, although the lack of effect on litter size implies that there was not a large increase in preimplantation loss. Secondly, as was previously conveyed to the sponsor, the doses selected were not adequate, given the absence of toxicity, the tolerability of doses up to 160 mg/kg in other rat studies, and the limited PK data (a comparison of the Cmax associated with 32 mg/kg obtained in a separate study to the human Css associated with the MRDD of 60 mg results in a ratio of 8; however, comparing Cmax and Css is not appropriate and when a mg/m^2 comparison is made the ratio is only 4.3). Lastly, an insufficient number of treated animals were evaluated; in particular, there were only 7 LD, 11 MD, and 8 HD C-sections.

The following is Dr. Rosloff's review of the three generation study, dated Sept. 19, 1983.

Dosage:
M: 24 at 0, and 12 at 1, 6, or 20 mg/kg/day, by gavage, from 10 weeks premating through mating.
F: 48 at 0, and 24 at 1, 6, or 40 mg/kg/day, by gavage, from 3 weeks premating through sacrifice on day 20 of gestation (1/2 F) or through day 21 PP (remaining F).

Mating ratio: 1M: 2F

Strain: Wistar/Af/Han/MOL

Results:
1) Observed signs in parents
   No drug effects

2) Parent mortality
   1 MD M (day 53 of premating)
   2 HD F (day 13 of mating and day 5 of gestation)

3) Parent bodyweight
   No drug effects

4) Mating/conception rate
   [Note inserted by Dr. Robin Huff during NDA review: In a submission subsequent to the original IND it was clarified that in fact there were no drug effects on mating or conception; the conception rate was 73, 67, 86, and 87% in controls, LD, MD, and HD, respectively. The confusion arose because the data originally presented did not include 11 females in which no vaginal plug was found, but which were later determined to be pregnant.]

a) Decrease in percent of M which mated both F at MD and HD (92, 83, 67, 50% in C, LD, MD, HD, resp.). (The proportion of these matings which were successful was not given.)

b) Decrease in percent of pregnant F at MD and HD (96, 92, 79, 75% in C, LD, MD, HD, resp.). (Another table shows no drug effect on conception rate. Clarification will be requested.)

c) No significant effect on time to mating (presumably calculated only for those which did mate.)

5) Length of gestation
No drug effect

6) Results in dams sacrificed day 20 of gestation
No drug effects on number of fetuses, litter size, fetal weight, gross external or visceral fetal abnormalities, or implantations. Resorptions were decreased at HD but not statistically significantly. Skeletal exam (Alizarin Red staining) showed no drug effects (# examined: 67, 23, 39, 44 in C, LD, MD, HD, resp.).

7) Results in dams allowed to deliver
[Note inserted by Dr. Robin Huff during NDA review: The 21% decreased survival of HD pups referred to below reflects pups killed by dams, not decreased pup viability.]
No drug effects on litter size, number of live fetuses, or (through day 21 PP) pup weight. Pup survival was slightly decreased at HD. Development of hearing response and eye opening was slightly delayed at HD, as was cliff avoidance at all doses. Other parameters were unaffected (unfolding of pinnae, static righting reflex, eruption of lower incisors, orientation by smell, righting reflex, visual test).

8) Reproductive performance of F1 generation
(2/sex/litter/group were used [untreated]. Mating [1M: 1F] took place on day 42-56 of age. Numbers of rats were: 24, 16, 12, 11 M and 25, 18, 14, 11 F in C, LD, MD, HD, resp. On day 20 of gestation, all F were sacrificed.)

a) No abnormal behavior.
b) No drug effect on maternal weight gain, aside from slightly decreased weight at HD on days 0 and 6 of gestation.
c) Conception rate was slightly (but NS) decreased at HD (64% vs. 84% in control).
d) No drug effects on litter-size, grossly abnormal fetuses, fetal weight, implantations, resorptions.
e) Skeletal exam (Alizarin stain; # examined: 70, 49, 36, 28 in C, LD, MD, HD, resp.)
Increased incidence of "weak skull ossification" and (at HD only) wavy ribs, said to be possibly due to fetal immaturity.

1) weak skull ossification in 3/70, 16/49, 5/36, 7/28 C, LD, MD, HD, resp.
2) wavy ribs in 6/70, 3/49, 2/36, 5/28 C, LD, MD, HD, resp.

f) Head exam (free hand slicing; # examined: 122, 87, 45, 36 in C, LD, MD, HD, resp.)
No abnormalities.

B. Segment II Rat Studies (11F/852; 182F/852; 206F/852) GLP, QA

In the original IND the sponsor submitted a Segment II study in which no drug effects were seen; however, the doses (0.8, 4.8, and 32 mg/kg) were insufficient for the same reasons discussed above for the three generation study. To correct this deficiency the sponsor subsequently submitted a second Segment II study (11F/852) that used doses of 32, 56, and 112 mg/kg, and in which cardiovascular and skeletal abnormalities occurred at the HD. A third Segment II study (182F/852), also with doses of 32, 56, and 112 mg/kg, revealed an increase in visceral and skeletal anomalies at the HD. Dr. Rosloff's original reviews of the latter two studies are excerpted below. Another Segment II study (206F/852) has also been submitted in which no notable findings are reported by the sponsor; however, the HD was only 56 mg/kg, the previously established NOEL.

Study 11F/852, excerpted Dr. Rosloff's April 14, 1989, review (cited tables and figures have not been included)

We had previously suggested (Pharmacologist review of 2/8/85; letter to firm of 3/11/85) that the firm perform a second segment II study in rats since the doses used in the first study (HD=40 mg/kg) appeared to be too low; also, nonstandard methodology was used. Doses in the new study were as follows:

> 22F at 0, 40, 70, and 140 mg/kg/day, by gavage, days 6-15 of pregnancy. (Strain; Charles River)

Dams were sacrificed day 20 of pregnancy. All fetuses were examined externally, 1/3 by Wilson free-hand sectioning technique, and 2/3 by Alizarin staining (following internal dissection). Results are summarized as follows; appropriate figures and tables from the submission are attached:

1) Observed signs:

   LD - salivation (N=2)
   MD - salivation, pupillary dilation, lethargy (N=5, 8, and 1 resp.)
   HD - same as above, seen in most animals

2) Dam bodyweight:

   Transient decreased gain at LD: transient weight loss at MD and HD.
   (These effects were seen during the first day or two of dosing only, after which rate of gain was similar to or greater than that of controls).

3) Dam food consumption:

   Decreased at MD and HD (approx. 90 and 80% of control, resp., during treatment period).

4) Increase in post-implantation loss (14% vs 3% in control), mainly due to increase in late resorptions, at HD.

5) Fetal weight decreased at HD (87% of control).

6) Fetal exam (See attached tables 6, 7, and 8 for numbers of fetuses examined and specific results, including historical control ranges).

There was an increase in abnormalities at HD, including the following:

a) External:
Increase in small fetuses (22% vs 2% in controls). Five fetuses had abnormalities, including umbilical hernia, shortened hindlimb digits, shortened or thread-like tail, imperforate anus, and pointed snout.

b) Free-hand sectioning:
A variety of abnormalities were considered drug-related. These abnormalities mainly affected the thoracic viscera, sternebrae, or extremities, and involved 19 of the 100 fetuses examined (from 11 of 22 litters). Thoracic visceral changes included cardiac septal defect. increased pericardial fluid above heart causing displacement of aortic arch and thymus, enlarged pericardial sac, small (thickened) contracted atrial walls, possible communication between pulmonary vein and right atrium, enlargement of left ventricle/reduction of right ventricle, reversal of esophagus and trachea, reduction in lung size. Sternebral abnormalities included sternebral cleft and apparent dorsal displacement of xiphisternum. Abnormalities of extremities included hindlimb brachydactyly, shortened tail, and constriction of tip of tail with or without associated hemorrhage. (Hydroureter and increased space between bodywall and organs thought to be due to fetal immaturity). For most of these abnormalities, the concurrent and historical control incidences were 0.

c) Skeletal Exam:
Increase in sternebral anomalies, esp. fused sternebrae, accompanied in 2 fetuses from separate litters by brachydactyly and shortened tail; 1 further fetus in a 3rd litter had a shortened tail but no sternebral anomaly. Overall, 29 of 177 fetuses (12 of 22 litters) were said to have been affected. A small number of rib and vertebral anomalies was also seen. Decreased ossification of several structures was considered to be due to fetal immaturity.

In summary, a variety of drug-related abnormalities were seen at HD, a dose which also caused maternal- and embryotoxicity. There was no clear effect at MD, a dose which caused slight maternal toxicity, although the sponsor did point out a few MD fetuses with abnormalities similar to those seen at HD (1 fetus had slight dorsal displacement of the xiphisternum and 1 had constriction at the tip of the tail; there was also an increased incidence of offset sternebrae [7% vs 2% in control, but incidence at HD was only 3%]).

Study 182F/852, excerpted from Dr. Rosloff's December 9, 1991, review (cited tables and figures have not been included). It is noted that the stability of the dosing solutions, which were prepared every fourth day rather than daily as in Study 11F/852, was not determined.

Dosage: 25 F at 0, 40, 70, and 140 mg/kg/day, by gavage, days 6-15 of pregnancy

Strain: Crl:CD (SD) BR VAF/Plus from

Dams sacrificed day 20 of pregnancy. Live young examined externally. Approx. 1/2 of fetuses in each litter examined for visceral abnormalities (Wilson method); remainder examined for skeletal abnormalities (Alizarin staining).

Results:

1) Observed signs in dams

   a) Salivation - all doses, D-R

   b) Red/brown vaginal discharge seen on day 15 in 2, 10, 10, and 8 dams in C, LD, MD, and HD, respectively.

2) Dam bodyweight

   Slight, transient weight loss at MD and HD and equivocally at LD. (See figure and table, attached.)

3) Dam food and water consumption

   Food consumption decreased at all doses, D-R. Max. effect days 6-8 of pregnancy at HD, 75% of control. (See attached table). Water consumption increased at HD

4) Litter data (See attached table)

   a) Post-implantation loss slightly increased at HD (9.1% vs. 3.4% in control, attributed to increases in both early and late deaths), but no decrease in live litter size.

   b) Fetal weight decreased at HD (92% of control).

5) Fetal exams (See attached tables) (Incidence values shown below are for fetuses).

   a) External exam - Results not shown

   b) Visceral exam

      Total incidence of visceral anomalies increased at HD (23% vs 12% in control). Individual findings contributing to this included abnormal lobation of liver and reduced thyroid. (The former was also equivocally increased at MD, especially when autopsy findings from skeletal exam pups are taken into account).

   c) Skeletal exam

      1) Total skeletal anomalies increased at HD (36% vs 18% control)

      2) Total variant sternebrae increased at HD (93% vs 74% in control), mainly due to unossified sternebrae (74% vs 45% in control).

      3) Flattened/connected sternebrae and dorsoventral distortion of the sternum slightly increased at HD (5% and 2%, respectively vs 0% for both abnormalities in both concurrent and historical controls).

      4) Irregular ossification of vertebral elements increased at HD (10% vs 5% in control).

      5) One less thoraco/lumbar vertebra increased at HD (7%) and equivocally at MD (5%). (Concurrent and historical control = 0% and 0.5%, respectively).

6) The following were equivocally increased at HD (not statistically significant [analysis by consultant in submission of 11/7/91], but greater than historical control): cervical ribs (also at LD and MD), shortened/absent 13th rib (also at LD and MD), split sternum, irregular costal cartilage elements, and reduced ossification of cervical vertebral arches.

7) The report states that some of the above changes suggest "an anterior shift in development of the axial skeleton".

d) The overall incidence of malformations of any type was not clearly affected by drug (1.1, 1.3, 1.4, and 3.6% in C, LD, MD, HD, respectively). The types of malformations observed were variable.

C. Segment II Rabbit Study (205/852) GLP, QA

Study 205/852 (0.8, 3.2, 12.8 mg/kg p.o) conducted in New Zealand White rabbits generated results similar to those in a previously submitted Segment II rabbit study (0.8, 4.8, 16 mg/kg p.o.) that was reviewed by Dr. Rosloff. The following has been excerpted from Dr. Rosloff's September 19, 1983, review (cited tables and figures have not been included).

Dosage:

mated F at 0, 1, 6, or 20 mg/kg/day, days 6-18 of gestation, by gavage. (A 40 mg/kg group was also started but results were not evaluated since 7 of 8 died. The 20 mg/kg dose was added as a replacement. "HD" will be used to refer to the 20 mg/kg group.) Does were sacrificed day 29 of gestation. Live animals were examined externally, internally by dissection, and skeletally (Alizarin).

Strain: New Zealand White

Results:
1) Maternal

a) Observed signs/mortality
Excluding does which were not pregnant or which died due to dosing errors, deaths were as follows: 2/17, 6/16, 4/17, 4/16, and 7/8 in control, 1, 6, 20, and 40 mg/kg groups, resp. A clear association with treatment was evident in all 7 at 40 mg/kg and in 1 at 20 mg/kg; in these rabbits signs included mydriasis, nervous/aggressive behavior, convulsive episodes, dyspnea, and cardiac arrhythmias. In the remaining does, the association of deaths with drug could not be established although mortality incidence was greater than in controls.

b) Bodyweight
Decreased gain at LD and MD and slight loss at HD; these effects mainly seen early in dosing period; by termination weights were similar to controls.

c) Pregnancy rate
94, 91, 100, and 100% in C, LD, MD, HD, resp.

d) Number of does with viable young
13, 9, 12, 12 in C, LD, MD, HD, resp.

2) Fetal

a) Total litter loss, pre- and post-implantation loss
No drug effect (slightly greater in controls, but not statistically significant).

b) Litter size
Slightly increased in all drug groups (related to decreased number of implantations and increased post-implantation loss in controls)

c) Fetal weight
No drug effect

d) Fetal exam
(Number examined: 86, 77, 100, 101 in C, LD, MD, HD, resp.)

1) Major malformations
Seen in 0, 1, 2, 1 in C, LD, MD, HD, resp. No pattern suggesting relationship to drug.

2) Minor anomalies
Seen in 6, 0, 3, and 14% of fetuses in C, LD, MD, HD, resp., at gross autopsy. (Half of the anomalies at HD seen in 1 litter, where pups showed signs relating to lethargy, discolored liver, enlarged atria, and poorly expanded lungs. Degeneration of the placenta was also observed suggesting these findings were not directly due to drug effects on the fetus.) There was no drug effect on incidence of minor anomalies seen at skeletal exam.

3) Skeletal variations
Incidence of extra ribs increased at MD (60% vs. 34% in C); increased at HD (48%), but no statistically significant. The incidence of variant sternébrae was increased in all groups. This was not dose-related and none of the changes was statistically significant. The nature of the variations was not given.

D. Segment III Rat Studies

An initial study (619/65, 732/65) was conducted dosing dams from GD17 to PD21 with 0.8, 4.8, and 32 mg/kg. In this study the effect on reproduction of the F1 generation was not evaluated (F1 pups were necropsied on PD 21), and the number of litters per group was insufficient (9, 9, 11, and 8 in control, LD, MD, and HD, respectively). As no substantial toxicity occurred in this study, the doses were increased to 16, 32, 64, and 128 mg/kg in a second study (192F/852) in which animals were dosed from GD17 to PD7. Again, this study did not examine the effect of treatment on reproduction of the F1 generation (pups were killed on PD7), and the number of litters per group was insufficient (6, 7, 8, 7, and 8 for control and increasing doses, respectively). In this study, maternal toxicity (decreased BW gain and salivation) occurred at 64 and 128 mg/kg, as did a decrease in pup viability (pup survival rate was 50% at 68 mg/kg and 0% at 128 mg/kg; the effect at 64 mg/kg was the consequence of underdeveloped maternal mammary papilla, whereas stillbirths also contributed to the effect at 128 mg/kg. In a third study (200F/852), dams were dosed from GD17 to PD21 with 0, 4.8, and 32 mg/kg. This study did examine the effect of treatment on reproduction of the F1 generation, and the HD resulted in mammary papillary immaturity that in turn compromised neonate survival. As this is the only study to examine an

adequate number of litters and to investigate the reproductive capabilities of the F1 generation, it is reviewed in more detail below.

Sprague Dawley (CrJ:CD/SPF) rats - 4.8, 12.8, 32 mg/kg p.o. gavage (batch 1232)
23 - 24 F/gr

HD BW gain from GD17-20 was 27% less than control; however, this did not impact GD20 total BW. Papillary immaturity observed in 4 HD F on PD1, 2 HD F on PD2, and 1 HD F on PD 3 - 6 likely caused the 4 total litter losses that occurred in HD animals. The litter losses culminated in a 74.6% survival index (v. 96.5% control). One total litter loss also occurred at the LD and there was one stillborn HD litter. Interestingly, although stillbirths were not increased in the MD group, defects, some of which were previously identified in teratology studies, occurred in MD stillborn fetuses; 1 fetus had ventricular septal defect, 1 had persistent atrioventricular canal, 1 had abnormal lobation of the liver, and 1 had abnormal lobation of the lung.

Birth weight of HD pups was decreased and weight remained depressed through day 70 in M and day 35 in F. Incisor eruption was delayed in HD animals; on day 11, eruption had occurred in 16% of HD fetuses v. 38% of controls, but by day 14, eruption was 100% in all groups. Other developmental milestones and reflexes were unaffected by treatment. Reproductive performance of the F1 generation was unimpaired, and there was no treatment effect on F2 fetal weight or external malformations.

In light of the effect on maternal mammary papilla that effectively limited dosing by decreasing pup viability, it would have been advisable to have conducted a study in which higher doses were administered on GD17-20 and pups were cross-fostered.

VI. Genetic Toxicology

A. Ames Test (204F/852) GLP, QA

Batch 1211

Strains TA98, TA100, TA1535, TA1537, WP2uvrA

Concentrations

range-finding:	50, 150, 500, 1500, 5000 µg/plate
main study:	156.3, 312.5, 625, 1250, 2500, 5000 µg/plate
reproducibility test:	as for main study without 156.3 µg/plate
additional tests 1 and 2:	1000, 1500, 2000, 2500, 3000 µg/plate

Results The 5000 µg/plate concentration was cytotoxic for all strains. Although in the main study there was no evidence of mutagenicity, mutagenic effects on the TA98 and TA1537 strains detected in the range-finding study were pursued in three other experiments, the results of which are summarized in the table below. It is concluded from the data that citalopram is mutagenic in the Ames assay. (Because the 3-fold increase in TA1537 revertants cited by the sponsor in the range-finding study occurred only as a result of an atypically low control replicate, it is not included in the table.)

Study	Revertant Increase (fold)			
	TA98		TA1537	
	-S9	+S9	-S9	+S9
range-finding	2.7 (1500) cytotoxic (5000)	-	cytotoxic (5000)	-
reproducibility	2.1 (1250, 2500) cytotoxic (5000)	-	2.4 (2500) cytotoxic (5000)	-
additional 1	2.6 (1500) 3.8 (2000) 4.6 (2500) cytotoxic (3000)	2.5 (2500) cytotoxic (3000)	2.0 (2000) cytotoxic (2500, 3000)	-
additional 2 (-S9 only)	2.6 (1500) 2.9 (2000) 4.2 (2500) cytotoxic (3000)	-	cytotoxic (≥2000)	-

The effective concentration (µg/plate) is given in parentheses.

B. Chromosomal Aberration Test in Human Lymphocytes (143F/852) GLP, QA

Batch 118-1

Concentrations

Exp 1:	-S9: 48.0, 68.6, 98 µg/ml	+S9: 233.6, 333.8, 476.8 µg/ml
Exp 2 (20 hr):	-S9: 102.4, 128, 160 µg/ml	+S9: 251.7, 314.6, 393.2 µg/ml
(44 hr):	-S9: 102.4 µg/ml	+S9: 491.5 µg/ml

Results

Treatment was for 3 hr under +S9 conditions and until harvesting at 20 hr (exp 1 and 2) and 44 hr (exp 2) under -S9 conditions. In experiment 1, no increase in chromosomal aberrations occurred. A statistically significant increase in polyploid cells occurred under +S9 conditions, but results exceeded the historical control range in only 1 replicate for the low and high concentrations, and this finding was not reproduced in experiment 2. The highest concentration tested reduced the MI by 49 and 63% under + and -S9 conditions, respectively. It is noted that a noncytotoxic concentration was not tested under -S9 conditions; the lowest dose reduced the MI by 35%. In experiment 2, results were also negative. The highest concentration used for the 20 hr harvest reduced the MI by 50 and 59% under + and -S9 conditions, respectively. At 44 hr the MI was reduced 43 and 52% under + and -S9 conditions, respectively. Although there was a statistically significant increase in aberrations at 44 hr under +S9 conditions, results were within the historical control range. It is concluded that citalopram is not clastogenic in the human lymphocyte assay.

C. Chromosomal Aberration Test in Chinese Hamster Lung Cells (331F/852) GLP, QA

Batch 1211

Concentrations

Test 1:	-S9: 4.8, 9.5, 19.0 µg/ml (24 and 48 hr treatment)
Test 2 and 3:	- and +S9: 110, 220, 440 µg/ml (6 hr treatment + 18 hr)
Test 4:	- and +S9: 55, 110, 220, 440 µg/ml (6 hr treatment + 18 hr)

It is noted that a -S9 positive control was included only in test 1.

Results

Concentrations that inhibited cell growth by 50% were determined to be 19 µg/ml when citalopram treatment was for 48 hr under -S9 conditions, and 440 µg/ml when treatment was for 6 hrs under +S9 conditions. Results were negative at the low concentrations used in test 1; however, cytotoxicity was not established for the 24 hr treatment regimen, and the 48 hr regimen, for which cytotoxicity was established, was atypically long. In test 2, 440 µg/ml produced chromosomal aberrations under both - and +S9 conditions (-S9: 13 v. 1%; +S9: 7 v. 0%). In test 3, cytotoxicity prevented evaluating the 220 and 440 µg/ml concentrations under -S9 conditions and the 440 µg/ml concentration under +S9 conditions. However, at the highest evaluable concentrations, aberrations were increased (-S9: 3 v. 0.5%; +S9: 2.5 v. 0.5%), exceeding the historical control range for -S9 conditions , but not for +S9 conditions . In test 4, interfering cytotoxicity occurred as in test 3, and increased aberrations were seen at the highest evaluable concentration under +S9 conditions (11 v. 0.5%). In test 4 only, an increase in polyploidy was

seen at the highest evaluable concentration under -S9 conditions and the two highest evaluable concentrations under +S9 conditions (-S9: 2.5 v. 0.5%; +S9: 1.25 and 2.25 v. 0.37%). Results from the 4 tests are summarized in the table below. It is concluded that citalopram is clastogenic in Chinese hamster lung cells.

S9	Genotoxicity (+ or -)			
	Test 1	Test 2	Test 3	Test 4
-	- (inadequate test?)	+	+	-
+	NA	+	+ w/i historical	+

D. HPRT Mutation Test in Mouse Lymphoma Cells (134F/852) GLP, QA

Batch 118-1

Concentrations Exp 1: - and+S9: 1.6, 5.0, 15.8, 50, 158 µg/ml
Exp 2: -S9: 6.2, 12.5, 25, 50, 100 µg/ml
+S9: 12.5, 25, 50, 100, 150, 200 µg/ml
Exp 3 and 4: - and+S9: 6.2, 12.5, 25, 37.5, 50, 62.5, 75, 100, 125, 150 µg/ml

Results In all experiments, cells were treated for 2 hrs, whereas OECD recommends 3 - 6 hr. In experiment 1 there was no evidence of mutagenicity; however the assay did not meet OECD standards for cytotoxicity; under -S9 conditions relative survival dropped from 90 to 1% between the two highest concentrations and under +S9 conditions relative survival was 82% at the highest concentration. In experiment 2, which did approximate OECD stipulated cytotoxicities, positive responses were observed under -S9 conditions at 12.5 and 25 µg/ml, but not 50 or 100 µg/ml and under +S9 conditions at 25, 50, and 100 µg/ml, but not at 150 and 200 µg/ml (note: relative survival was only 8%, slightly less than the recommended minimum of 10%, at 200 µg/ml). The positive results seen under -S9 conditions were not reproducible in experiments 3 and 4; however, while results were negative under +S9 conditions in experiment 3, the two highest concentrations were positive in experiment 4 (note : relative survival was only 5% at the highest concentration, decreasing the reliability of this culture). In summary, the positive results that occurred under -S9 conditions occurred only at intermediate concentrations (i.e., were not concentration-dependent) and were not reproducible. The initial positive results
seen under +S9 conditions were not concentration-dependent, and in an additional experiment a positive result (4.4-fold increase in MF) occurred only at the highest analyzable concentration. Results of the HPRT mutation test are equivocal.

E. Unscheduled DNA Synthesis in Rat Hepatocytes (142F/852) GLP, QA

Batch 118-1

Doses 160 and 800 mg/kg p.o.; 5 M analyzed/gr

Results Results were negative in cultures prepared at 4 and 12 hrs after dosing. Deaths occurred at the HD, which attests to sufficient dosing, but 5 animals survived for hepatocyte preparation. It is noted that only 50 cells/animal were examined whereas OECD recommends 100.

F. In Vivo Micronucleus Assay - NMRI Mice (561F) GLP, QA

Batch XVIII

Doses 125 and 250 mg/kg p.o.; 5 F/gr (24, 48, and 72 hr)
250, 375, and 500 mg/kg p.o.; 5/s/gr (24 hr)

Results Results were negative; however, it is noted that only 500 PCE's were examined per animal, rather than the 2000 stipulated by OECD. Apparently the PCE/NCE ratio was not determined, so bone marrow toxicity cannot be assessed. General toxicities are not described, but the HD of 500 mg/kg may have been sufficient if the death of one F was treatment-related (it is not stated if this was the case).

G. In Vivo Micronucleus Assay - CD-1 Mice (133F/852) GLP, QA

Batch 118-1

Doses 81.7, 163, 327 mg/kg p.o.; 5/s/gr (all groups 24 hr, HD also 48 and 72 hr)

Results Citalopram was not clastogenic at any dose. The HD approximated 75% of the LD50 and one HD died prior to the 48 hr sampling. The sponsor notes that some bone marrow toxicity may have occurred in MD and HD males because the PCE/NCE ratio (MD: 0.58; HD: 0.78) was below the historical control range; however, the HD ratio was barely outside the control range It is noted that only 1000 PCE/animal were examined, rather than the 2000 stipulated by OECD.

VII. Carcinogenicity Testing

A. Mice, 18 month dietary mix (22/852) GLP, QA

NMRI Mice - 40, 100, 240 mg/kg (batch 1061)
50/s/gr with 2 control groups (+ 40/s at 240 mg/kg for toxicokinetics)

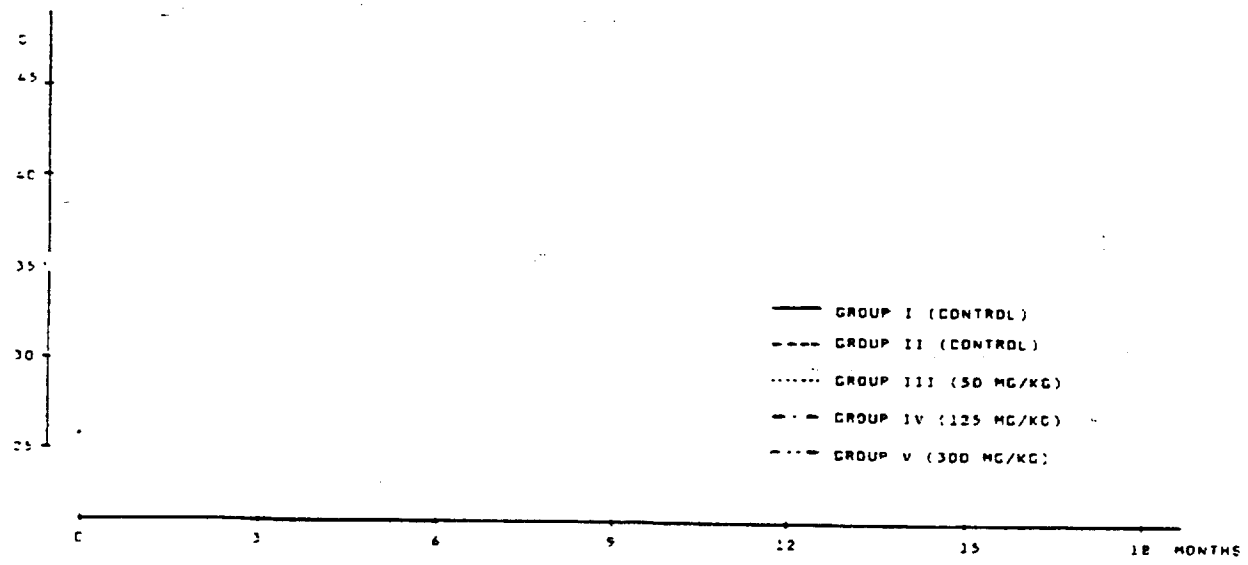
Note: Comparisons are made to the control group with the highest incidence of any given finding.

Mortality — Mortality in HD M significantly exceeded control mortality (58 v. 28%). The sponsor indicates that the primary cause of death/premature sacrifice was obstructive uropathy resulting in distension of the urinary bladder and uremia.

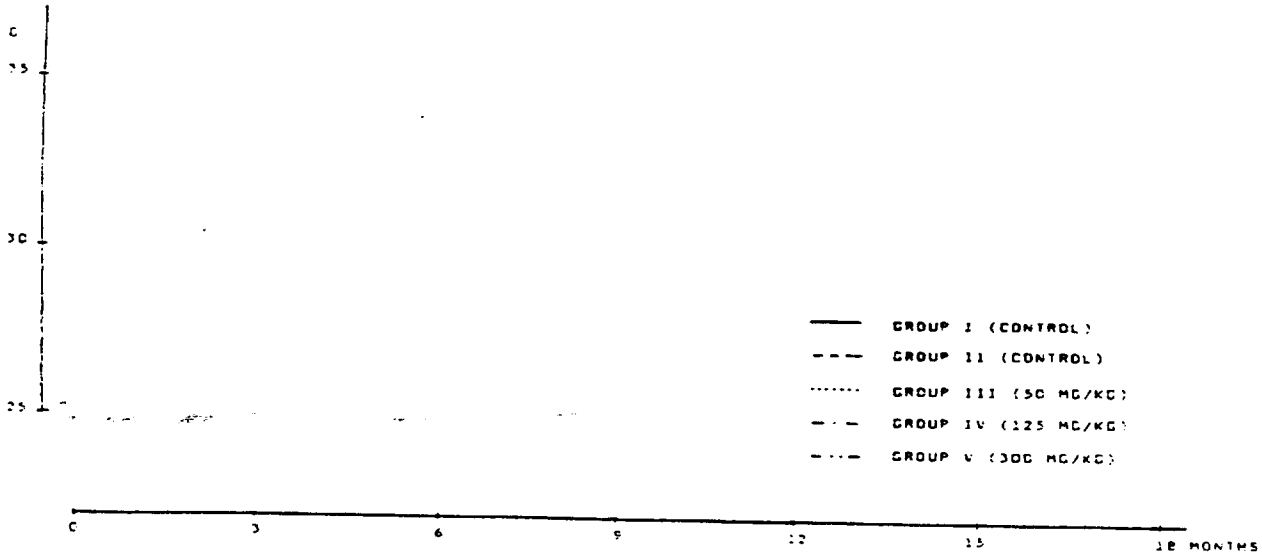
Clinical Signs — The incidence of tonic-clonic convulsions increased in MD and HD males and HD F. This increase was noted as of week 38 in HD M and F, and as of week 54 in MD M, and the proportion of HD animals affected reached 35 - 50%. The control incidence was approximately 10% late in the study. In the latter portion of the study the incidence of sedation increased in HD M (20 v. 3% in control). Palpation revealed a dose-related increased incidence of urinary bladder distension in treated males that occurred as of week 50 and was associated with concomitant constipation in the HD group.

Body Weight — (See sponsor-supplied graphs below.) LD and MD M and LD F had increased body weights relative to control throughout the study (16, 9, and 6%, respectively). In HD M and F, BW was decreased relative to control. Food consumption was increased dose-dependently in all treated groups.

Males



Females



Toxicokinetics Blood, brain and liver samples were taken from 3 - 5 HD mice/sex at 2, 14, 26, 34, 42, 51, 60, and 69 weeks. Blood was also taken from 5/sex for each dose group at study termination. In HD animals, plasma concentration increased after 2 weeks (more gradually for F than M), and declined during the latter half of the study. Mid-study HD plasma levels reached ~9 μM, and terminal concentrations were ~4 μM. At most time points, plasma concentrations in F were slightly less than in males. After 2 weeks, levels of the desmethyl and didesmethyl metabolites were fairly constant at ~3.5 and ~1 μM, respectively. Based on blood collected at termination from all groups, plasma levels of citalopram and both metabolites increased in a dose-linear manner, except for citalopram and the desmethyl metabolite in males, which increased in a greater than dose-linear manner (see sponsor-supplied graphs below).

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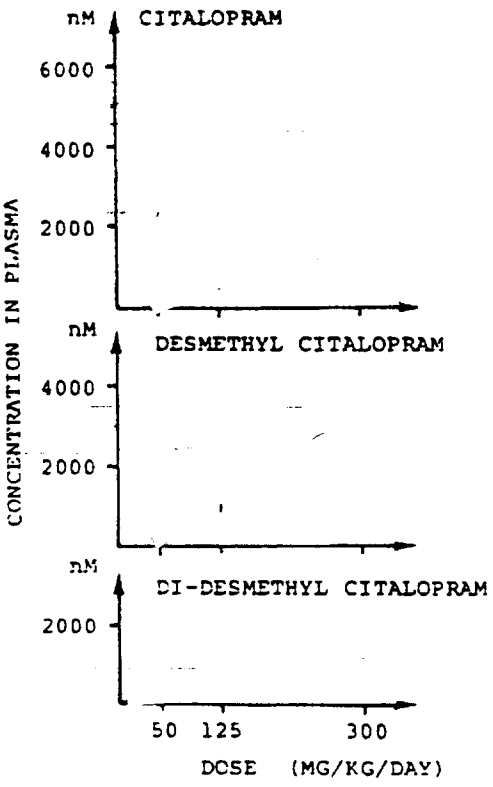

Plasma levels of citalopram correlated with levels found in both brain and liver. Brain content of citalopram was generally around 25 µg/g, with levels of the desmethyl metabolite being 10-fold less. Liver content of citalopram was generally around 150 µg/g, with levels of the desmethyl and didesmethyl metabolites being approximately 2 and 4-fold less, respectively. Levels of all three compounds tended to be slightly higher in the livers of F.

Pathology

Treatment with citalopram did not increase the incidence of any neoplastic finding.

Non-neoplastic findings are summarized in the table below. There was an increased incidence of renal hydronephrosis and pelvic dilation in MD and HD M, probably related to the increased incidence of urinary bladder distension. It is hypothesized by the sponsor that formation of urethral plugs by solidified ejaculates initiated the bladder and nephropathology. While an effect of citalopram on ejaculation has not been investigated, the 5-HT depleting drugs PCPA and reserpine have been demonstrated to affect ejaculation. Other non-neoplastic findings included greater incidences of empty stomachs, liver necrosis, and liver congestion in HD M. The only notable finding in females, was that the incidence of cystic endometrial hyperplasia was increased in LD and MD F (but not HD F).

Non-neoplastic Finding	Incidence in Males			
	Control	LD	MD	HD
Kidney				
hydronephrotic	1/50	1/50	**5/50**	**7/50**
renal pelvic dilation	12/50	13/50	**22/50**	17/50
Urinary Bladder				
distended, macro	5/50	**10/50**	**23/50**	**29/50**
distended, histo	4/48	**8/50**	**31/50**	**34/49**
urethral plug	2/50	2/50	2/50	**7/50**
hemorrhagic	0/50	2/50	1/50	**5/50**
mononuclear cells	2/50	1/50	**7/50**	**5/49**
congestion	1/48	**4/50**	**3/50**	**6/49**
Stomach, empty	1/50	1/50	4/50	**7/50**
Liver				
necrosis	3/50	0/50	1/50	**9/50**
congestion	9/50	2/50	6/50	**31/50**
	Incidence in Females			
Uterus				
cystic endometrial hyperplasia	0/49	**14/50**	**11/50**	0/48

Summary

Citalopram was not carcinogenic in NMRI mice, when administered over an 18 month period. (Generally a 2 yr study is required; however, it was communicated to Forest that 18 months might be acceptable if the rat study was negative and the mouse study was not suggestive.) Pathology in males was limited to a dose-related increased incidence of urinary bladder distension, associated with other bladder and renal pathology, and liver necrosis and congestion at the HD. The only notable pathology finding in females was an increased incidence of uterine cystic endometrial hyperplasia in LD and MD animals.

Based on the convulsions that occurred in of HD M and F during the latter half of the study, the increased mortality observed in HD M, and the decrease in BW relative to control that occurred in HD M and F, the HD is acceptable as an MTD. Positive genetic toxicology findings preclude using PK data to justify dose selection.

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ON ORIGINAL

B. Rats, 2 yrs dietary mix (748F) GLP, QA

COBS WI (Wistar) rats - 8, 24, 80 mg/kg (batch 1031)
50/s/gr with 2 control groups

Notes: 1) Comparisons are made to the control group with the highest incidence of any given finding.

2) The dosage achieved was generally less than nominal from week 55 for HD males and from week 35 for HD F.

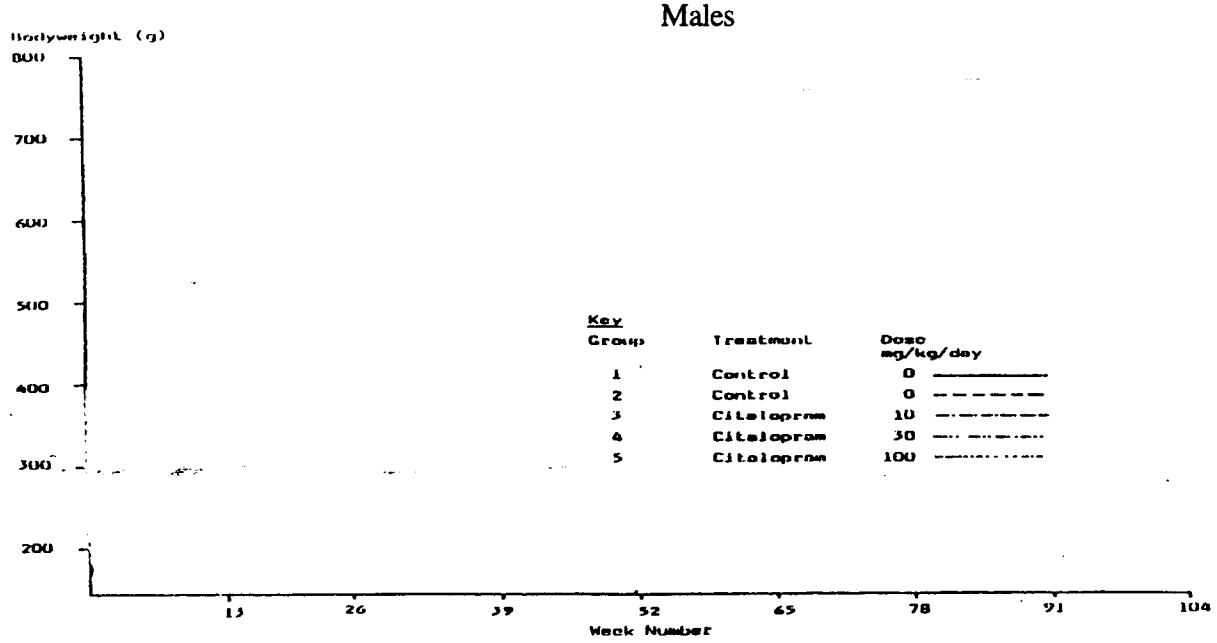
3) Degradation of citalopram to the N-oxide occurred in the feed, which was prepared on a weekly basis. The precise extent of degradation at the time of use is not known for most samples because analysis was delayed generally 2 - 8 weeks, by which time N-oxide accounted for up to 29% of total drug. Stability studies conducted with diet containing 200 ppm citalopram revealed a 3% degradation to N-oxide in 1 week; however, all dose groups used diets containing >200 ppm citalopram. Since degradation rate was shown to be inversely related to drug concentration (50 and 200 ppm tested), presumably the N-oxide should account for <3% of the administered doses. However, week 104 LD diet samples (~300 ppm) that were analyzed 1 week after preparation contained 6% N-oxide, and week 91 samples analyzed 2 weeks after preparation contained 15% N-oxide, values that exceed the predicted degradation.

Mortality — Mortality was decreased in HD M and F, with a delay in onset in HD F. Mortalities were 28% (HD M), 12% (HD F), 38/44% (Con M), and 32/38% (Con F).

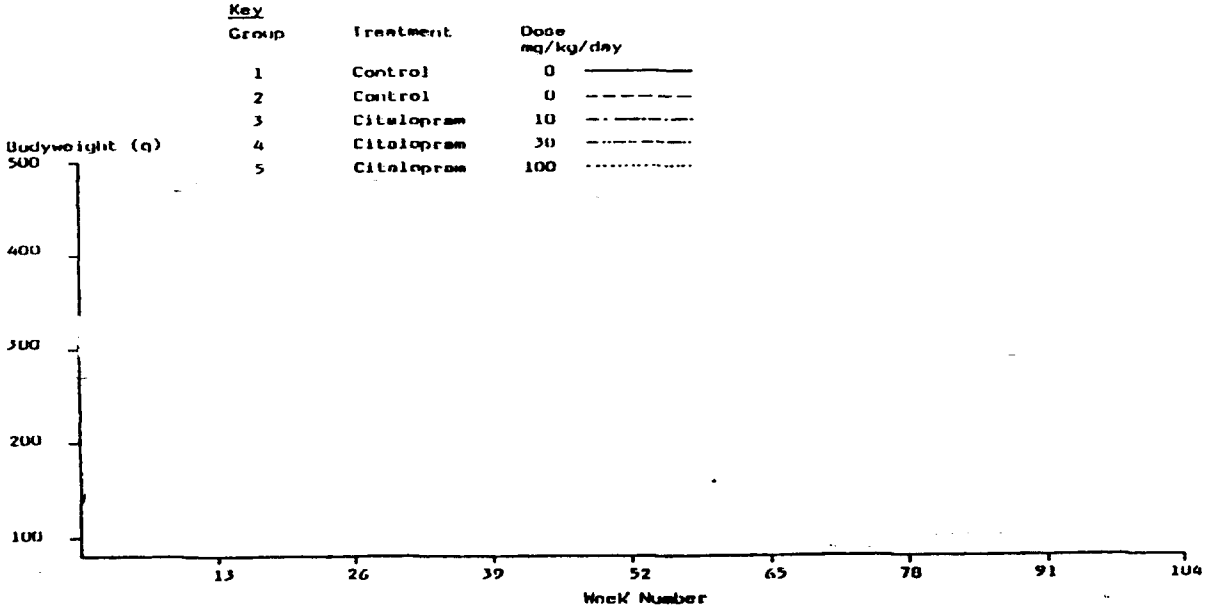
Clinical Signs — Increased tail rigidity leading to a permanent "corkscrew" appearance occurred as of week 12 in HD animals and later in the study in MD animals. Although periods of hyperactivity that involved muscle spasms, violent twitching, rearing, and rapid respiration occurred, the incidence was similar in control and treated groups.

Body Weight — (See sponsor-supplied graphs below.) In HD M and F, BW was reduced relative to control by › at week 13 and progressed to 30% below control by study termination. There were › decreases observed in MD M throughout the study and decreases in MD F gradually progressed to . Food consumption was decreased in HD M throughout the study and after week 13 in HD F. Food consumption was also decreased in MD animals after week 13, in M and in F.

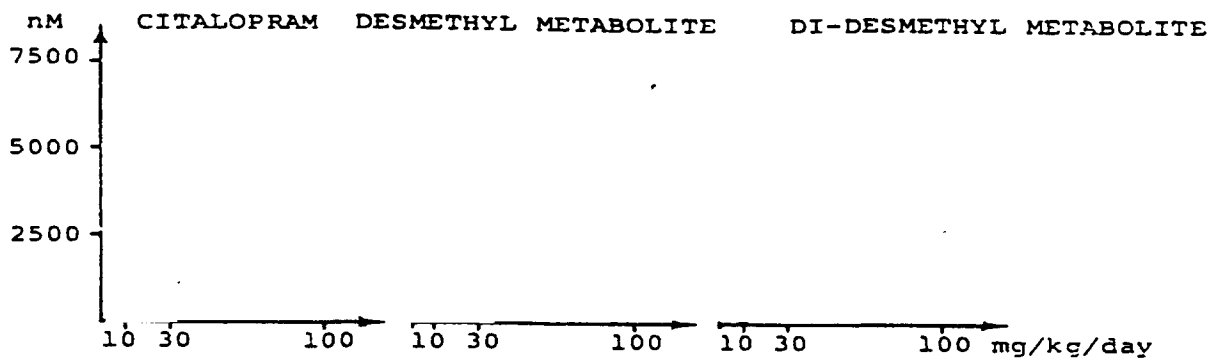
Males



Females



Toxicokinetics Samples were taken from all survivors at study termination. Plasma levels of citalopram and the demethylated metabolites increased linearly with dose (see sponsor-supplied graphs below). Levels of metabolites, but not citalopram tended to be slightly less in F than M.



Comparing terminal plasma concentrations to those determined during the 1 yr rat study, it appears that the concentration of citalopram and metabolites increased over time.

Pathology

Notable neoplastic findings were limited and are summarized in the table below. Absence of an increased incidence at the HD was discounted due to the excessive decrease in BW in HD M and F.

Tumor Type	Incidence							
	Control		LD		MD		HD	
	M	F	M	F	M	F	M	F
Kidney carcinoma	0/50	0/50	0/50	0/50	0/50	**2/50**	0/50	0/50
Small Intestine carcinoma	0/50	0/50	**4/50**	1/50	**2/50**	1/50	0/50	0/50
Mammary Tissue adenofibrosarcoma	0/50	0/50	0/50	0/50	0/50	**2/50**	0/50	0/50
Skin squamous papilloma	1/50	0/50	1/50	0/50	**3/50**	0/50	0/50	0/50

Hyperplasia was notably increased only in lymph nodes. Other non-neoplastic lesions included an increased incidence of foci in the lungs of MD and HD M and of macrophage accumulation in the lungs of MD M and HD M and F. There were several ophthalmologic findings, including increased retinal degeneration in HD M and F. Sciatic nerve degeneration was enhanced in MD F and HD M and F. The incidences of liver necrosis and mammary gland duct proliferation were increased in HD M, as were the incidences of small, flaccid testes and focal testicular calcification. Follicular cysts were more prevalent in HD F. Non-neoplastic findings are summarized in the table below.

Non-neoplastic Finding	Incidence							
	Control		LD		MD		HD	
	M	F	M	F	M	F	M	F
Lymph Node								
reactive hyperplasia (cervical)	10/50	12/50	14/50	19/50	15/50	**24/50**	14/50	7/50
reactive hyperplasia (mesenteric)	3/50	3/50	2/50	3/50	3/50	**8/50**	4/50	3/50
Lung								
foci	10/50	6/50	15/50	4/50	**19/50**	8/50	**31/50**	9/50
macrophage accumulation	16/50	7/50	18/50	6/50	**28/50**	11/50	**45/50**	**39/50**
Eye								
lens opacity (eye exam, wk 103)	4/32	6/35	7/31	2/34	**9/34**	4/36	**12/39**	1/45
corneal opacity (eye exam)	2/32	5/35	7/31	1/34	8/34	4/36	4/39	**11/45**
retinal degeneration	9/50	27/50	7/50	20/50	10/50	19/50	**20/50**	**35/50**
keratitis	1/50	1/50	0/50	0/50	**3/50**	0/50	**4/50**	0/50
Sciatic Nerve								
degeneration, minimal	16/50	5/50	11/50	8/50	26/50	**11/50**	9/50	**24/50**
degeneration, moderate	4/50	1/50	7/50	2/50	1/50	0/50	**15/50**	1/50
Liver								
centrilobular necrosis	2/50	0/50	1/50	1/50	0/50	1/50	**7/50**	1/50
multinucleated hepatocytes	0/50	0/50	0/50	0/50	**3/50**	**2/50**	0/50	0/50
Mammary Gland								
duct proliferation	1/50	3/50	0/50	4/50	0/50	1/50	**5/50**	1/50
Testis								
small, flaccid	16/50		11/50		8/50		**40/50**	
focal calcification	5/50		3/50		8/50		**24/50**	
Ovary								
follicular cyst		6/50		4/50		8/50		**16/50**

Summary

Citalopram showed limited carcinogenic potential in Wistar rats, showing only small increases in the incidences of small intestine carcinoma, kidney carcinoma, mammary adenofibrosarcoma, and squamous papilloma of the skin. It was the opinion of the Executive Carcinogenicity Assessment Committee that the overall incidence of small intestine carcinoma (8/200 treated animals [HD animals were excluded, see below] versus 0/200 controls) suggested a treatment-related effect on a tumor type that appears to be rare. Historical control data indicate a background range of 0/105 - 5/400, with the upper limit being 3.2-fold less than the incidence of small intestine carcinoma in citalopram-treated animals.

The HD had to be discounted when assessing carcinogenic potential because BW was decreased excessively relative to control (~30%). Reduced BW may have contributed to the increased survival of HD animals. The modest BW losses

experienced by MD animals suggest that this dose, or one between the MD and HD, should have been used as a HD.

Although the study indicates limited carcinogenic potential for citalopram, reliability of the results is complicated by the fact that citalopram was shown to degrade in the feed to the N-oxide (see Note 3, above). Thus animals may have been underdosed with citalopram, and the reduced BW used to define an MTD may have resulted from exposure to the N-oxide, rather than citalopram. The N-oxide has been identified in rat urine, and was described as contributing less than citalopram, the desmethyl metabolite, or the didesmethyl metabolite (no numerical results were presented). In no study was the N-oxide identified in rat plasma.

SUMMARY AND EVALUATION

Pharmacology

The antidepressant activity of citalopram is ascribed to its inhibition of serotonin reuptake. The reported K_i for uptake inhibition at the cloned transporter is 6.1 nM. *In vitro* selectivity for serotonin uptake inhibition over norepinephrine and dopamine uptake inhibition is ≥4-fold better with citalopram than with sertraline, paroxetine, fluvoxamine, fluoxetine, DCT, clomipramine, and amitriptyline. Inhibition of uptake is accomplished by citalopram binding to the high affinity imipramine site on the serotonin transporter. It is the (+)enantiomer that is active, and the (+)enantiomer of the monodesmethyl metabolite possesses 12 - 14% of the activity of the parent molecule.

Antidepressant activity of citalopram was demonstrated in multiple animals models. In the forced swimming mouse model, effective doses were 0.01 - 40 mg/kg s.c.; in the learned helplessness rat model, effective doses were 1 - 2 mg/kg b.i.d. i.p.; in the chronic mild stress rat model, 10 mg/kg i.p. was the only dose tested. In addition to exhibiting antidepressant properties, citalopram decreased the conditioned fear response in rats and increased exploratory behavior in both rats and mice.

No physical dependence on citalopram was demonstrated in rats, nor was any psychological dependence demonstrated in monkeys. Like other antidepressants, there was a pharmacological interaction of citalopram with monoamine oxidase inhibitors which block metabolic deamination of serotonin. Also like other antidepressants, citalopram decreased REM sleep in the cat. The cardiovascular effects of citalopram varied in different species. There were no ECG changes in rabbits given 20 mg/kg i.v. or in pigs given 5 mg/kg i.v.. A 5 mg/kg infusion produced transient (15 min) QRS broadening and premature beats in cats; this dose resulted in plasma concentrations of citalopram ~50 times that achieved in humans given the MRDD of 60 mg. Dogs were particularly susceptible to citalopram, with cardiovascular complications suspected in the deaths that occurred at 8 mg/kg during the 1 year oral study; on a mg/m^2 basis, this dose is only 3.6 times the human MRDD of 60 mg. In prior studies 10 mg/kg p.o. had produced tachycardia, labile heart rate, convulsions and eventually death. An i.v. study designed to investigate the deaths that occurred in the 1 year study revealed ventricular arrhythmias in dogs given 10 mg/kg/hr citalopram and QT prolongation in dogs given 2.5 mg/kg/hr DDCT, the didesmethyl metabolite. While DDCT levels are negligible in humans, DCT (the monodesmethyl metabolite) levels are 1/2 to 1/3 those of citalopram, yet the cardiovascular effects of DCT were not investigated. The results of the exploratory cardiovascular study in dogs are described in more detail below in the Cardiovascular Toxicity section.

Absorption, Distribution, Metabolism, and Excretion (ADME)

Absorption of citalopram in animals, as indicated by bioavailability, was specie and sex-dependent, but is good (approx. 80%) in humans. Cmax and AUC generally increased in a dose-proportional manner, and values tended to be greater in F than M animals. The half life of elimination is greater in humans than in animals, 37 hr versus 1.5 - 13 hr. Elimination of the didesmethyl metabolite takes even longer; the human $t_{1/2}$ is 100 hr. Distribution of citalopram was extensive, with Vd's of 25, 10, and 12 L/kg in rats, dogs, and humans, respectively. Gonads and pigmented tissues of rats retained citalopram 1 week after administration; $t_{1/2}$'s were 5.7, 16, and 41 days for gonads, eyes and skin, respectively. The retention by pigmented tissues indicates binding to melanin, a feature common to several antidepressants. Protein binding was 77, 71, 75, and 82% in mouse, rat, dog, and human plasma, respectively, and binding of the desmethyl metabolites was similar to binding of citalopram.

Citalopram is primarily metabolized to desmethyl and didesmethyl metabolites (DCT and DDCT); subsequent metabolism produces the propionic acid, and alternative metabolism produces the N-oxide. *In vitro* studies indicate that demethylation is performed primarily by CYP 2D6. Stereoselective removal of the second methyl group, or stereoselective excretion of the didesmethyl metabolite, is suggested by the 3:1 plasma ratio of + to - DDCT enantiomer. There was no interconversion of citalopram, DCT, or DDCT enantiomers when they were incubated in dog or rat serum *in vitro*; however, incubation was only at room temperature, not physiological temperature.

In both animals and humans, the majority of a citalopram dose is excreted as citalopram, DCT and DDCT. In rats, 43% of the dose was excreted in the urine and 35% in the feces within 48 hr, with most metabolites identified in both urine and feces. In dogs, significant concentrations of citalopram and metabolites were found in bile. In humans, 75% of a single 40 mg oral dose was excreted in urine and 10% in feces over a 14 day period. At steady state, after 4 weeks of treatment, 46% of the dose was excreted in urine (23% as citalopram, 19% as DCT, and 4% as DDCT). Hepatic elimination is substantial in humans as hepatic insufficiency increased the $t_{1/2}$ of citalopram from 37 to 83 hr.

General Toxicity

One year oral toxicology studies were conducted in rat and dog. The rat dietary study (32, 60, 120 mg/kg) is complicated by degradation of citalopram to the N-oxide, which has been identified as a minor metabolite in rat urine, but has not been identified in plasma. The precise extent of degradation is unknown because feed analyses were delayed. Clinical signs included occasional seizure-like activity in all treated groups. During the recovery period such activity also occurred in controls, but HD F exhibited a 3 - 4-fold increase in incidence. In addition to seizure-like activity, treated animals displayed increased tail rigidity as of week 12, such that tails took on a corkscrew appearance; the incidence and severity were dose-related. BW gain was decreased in all groups such that at week 52 BW was ~30, 18, and 6% less than control in HD, MD, and LD groups, respectively. After 50 weeks of treatment, HD M experienced mild anemia that gradually recovered. HD M also experienced leukocytosis, and the differential count was redistributed with an increase in neutrophils and a decrease in lymphocytes. A similar redistribution occurred in HD F, but the increase in WBC was less than in males. Lymphocytes were vacuolated in all HD animals, with a greater proportion of cells affected in M than F; earlier, at week 26, HD animals displayed varying degrees of vacuolation. A few MD animals displayed occasional vacuolation at week 50. Effects on WBC total and differential count lessened during recovery, but were still present after 12 weeks. There was no evidence of lymphocyte vacuolation after recovery. Throughout the study, several individuals experienced changes in clinical chemistry such as several-fold increases in ALT accompanied by ≤2-fold increases in AST. AP was increased 40% in HD F at the end of treatment, but returned to normal during recovery. Triglycerides, cholesterol, and HDL were decreased in HD M at 50 weeks and throughout recovery; similar decreases were seen in HD F, but only during recovery. Lipid analysis of male liver samples revealed a dose-related increase in total lipids and cholesterol, and a doubling of triglycerides in all groups, that abated after 12 weeks of recovery. Metabolism of citalopram to its demethylated derivatives has been shown to be ~2-fold greater in males than females and may have contributed to the sex-selective liver alterations; however metabolite levels in HD F exceeded that of LD and MD M, yet HD F did not exhibit hepatic lipid changes (or vacuolation, see below). Additionally, clinical chemistry findings may suggest a change in liver function; liver was one of the target organ identified, as described below.

Organ weight changes in HD animals generally reflected the significant decrease in BW. The increase in relative adrenal weight in HD M exceeded the increases in other organs, but was absent after 13 weeks of recovery. Target organs included the liver, which in nearly all treated M

contained ORO-positive fat and vacuolated hepatocytes. Furthermore, 5/20 HD F exhibited parenchymal inflammatory cell infiltration. Persistence of most effects cannot be evaluated because H and E histopathology was not performed on tissues of recovery animals. ORO-staining, which was examined in livers of recovery M, did persist through 4 weeks of recovery, but by 13 weeks control incidence and severity had increased to treated levels. In an ancillary study, the NOEL for increased ORO staining was determined to be 0.8 mg/kg, which provides less than the mg/m^2 exposure obtained in humans given the MRDD of 60 mg. In addition to liver pathology, interstitial inflammatory cell infiltration of the kidneys of M occurred in a dose-related manner. Lung pathology included perivascular accumulation of lymphocytes in all M treatment groups, and increased incidence and severity of macrophage accumulation in all M and F treated groups. Macroscopically, white areas were noted on the lungs of treated M, even after recovery. The incidence of thymus involution increased in treated M, but did have a high background incidence in controls. Testicular tubular atrophy and tubular calcification occurred in MD and HD M, and epididymal spermatozoa were reduced in HD M. To provide perspective when considering the above pathologies in relation to clinical dosing, mg/m^2 exposures achieved in LD, MD, and HD groups were 4, 8, and 16 times, respectively, the exposure achieved in humans given the MRDD of 60 mg. Plasma concentrations increased over time (AUC's were not determined); levels at 26 weeks (mid-study) were 2, 3, and 8 times the Css achieved in humans given 60 mg daily. Pharmacokinetic coverage was greater for both the mono- and didesmethylated metabolite.

In the 1 year dog study, doses of 1, 3, and 8 mg/kg were administered via capsule. Deaths of 5 HD animals occurred during weeks 17, 18, 27 (2), and 31, generally within 2 - 4 hr of dosing. Deaths were unheralded in that prior to death animals had been in good condition and there had been no definitive adverse ECG or clinical pathology findings. This is in contrast to the deaths that occurred at 10 mg/kg during weeks 3 - 7 of the 3 month study; these deaths were associated with convulsions, labile heart rate and restlessness. Retrospective analysis of ECG's recorded prior to dosing during weeks 6, 12, 25, 38, and 51, indicates that QT interval in HD animals was increased ~10% above controls, and that dogs that died tended to have higher values than survivors; however, values overlapped and the data do not conclusively implicate QT prolongation as the cause of death, particularly since recordings were made prior to treatment, whereas deaths generally occurred 2 - 3 hr after treatment. (Acute cardiovascular studies did not identify any drug-induced ECG changes at doses ≤10 mg/kg, although tachycardia and increased blood pressure were noted at doses ≥ 5 mg/kg. The number of animals examined was limited.) Other than the small effect on QT interval, drug effects were limited to mydriasis and salivation at the MD and HD, and increased thymus weight at the HD. For the first 15 weeks of treatment only, mydriasis in HD animals was accompanied by impaired light accommodation. Plasma levels increased between 4 and 29 weeks, stabilizing thereafter. Plasma levels measured early in the study were similar in HD survivors and dogs that later died. The C2hr (~Cmax) pharmacokinetic parameter reported for dog is not directly comparable to the Css reported for humans given the MRDD of 60 mg. If, however, this comparison is made using week 29 and forward data, dog/human exposure ratios are 0.3, 2.4, and 9.4 at the LD, MD, and HD, respectively. Ratios for the mono- and didesmethylated metabolites are greater. If comparisons are made on a citalopram mg/m^2 basis, ratios are 0.5, 1.4, and 3.6, respectively.

Cardiovascular Toxicity

In an effort to investigate the unexplained, sudden deaths that occurred in HD dogs during the 1 year study, cardiovascular function was monitored in dogs given acute, intravenous doses of citalopram and its didesmethyl metabolite. Dogs were given 10 mg/kg/hr citalopram, 2.5 mg/kg/hr didesmethyl citalopram (DDCT) or both. HR, BP, and cardiac histopathology were unperturbed by drug treatment. Convulsions occurred in both groups that received citalopram, as had been observed with oral doses ≥10 mg/kg. Ventricular arrhythmias secondary to CNS stimulation were

also observed in both groups that received citalopram. Prolongation of the QT interval was observed in both groups that received DDCT. In the combination group ventricular arrhythmias were fatal, leading the sponsor to conclude that deaths resulted from an interaction between the QT prolonging effect of DDCT and the centrally mediated arrhythmic effect of citalopram. When plasma levels were measured there were lethality thresholds for both citalopram and DDCT; exceeding both thresholds was associated with death, whereas exceeding only one was not. Lethal plasma concentrations of citalopram and DDCT were similar in this intravenous study and the 1 year oral study, and exceed concentrations achieved in humans by ≥3.5-fold.

Although the acute i.v. study offers one possible explanation for the deaths observed in the 1 year oral study, the hypothesis is not entirely consistent with the data from the 1 year oral study, nor have other possibilities been explored. For instance, while the long half-life of DDCT (1 - 2 days) would result in accumulation of DDCT over time and interactions of DDCT and citalopram would be expected to be delayed, it is not clear why deaths were delayed until week 17 because DDCT levels should have reached steady state by 2 weeks. Additionally, although lethal plasma concentrations were similar in the i.v. and oral study, the faster rate of concentration increase may also have contributed to the i.v. lethality. Lastly, the role of the monodesmethyl metabolite was not investigated and may be more relevant to humans, as plasma levels that of citalopram are achieved (by contrast, DDCT is barely detectable in human plasma). It is acknowledged that the human plasma levels of the monodesmethyl metabolite are ~8 times less than levels attained in dogs that died in the 1 year study.

Reproductive Toxicity

Reproductive toxicity studies were conducted in rats and rabbits. A three generation rat study was conducted in which the findings were limited to a slight delay in hearing development and eye opening in HD F1 pups, and an increased incidence of weak skull ossification in all treated F2 groups (weak ossification was ascribed by the sponsor to fetal immaturity; however, fetal weight was not affected). The doses of 0.8, 4.8, 16(M)/32(F) mg/kg p.o. were inadequate, as evidenced by the lack of maternal and fetal toxicity and the tolerability of doses up to 160 mg/kg in other studies. Furthermore the number of animals evaluated was insufficient; in particular, there were only 7 LD, 11 MD, and 8 HD F0 C-sections. Lastly, corpora lutea were not counted, making assessment of the effect of treatment on preimplantation events difficult. These issues are of particular concern because although other Segment II and III studies were submitted, the three generation study was the only one to incorporate a Segment I portion.

Evidence of teratogenicity was found in both rat Segment II studies that used doses of 32, 56, and 112 mg/kg. Mild maternal toxicity, including salivation, pupil dilation, and lethargy, were observed in most HD dams in study 11F/852, indicating that the selected dose was sufficiently high. Decreased BW gain was also observed, but only during the first two days of dosing. Post-implantation loss was increased from 3% in controls to 14% at the HD, mainly as a result of late resorptions. HD fetal weight was decreased to 87% of control. Abnormalities were increased at the HD, including the following cardiac aberrations: cardiac septal defect, increased pericardial fluid that displaced the aortic arch and thymus, enlarged pericardial sac, small thickened contracted atrial walls, communication between pulmonary vein and right atrium, and enlargement of the left ventricle/reduction of the right ventricle. There was also an increase in skeletal defects such as sternebral cleft, displacement of the xiphisternum, and fused sternebrae. In total 19/100 fetuses from 11/22 HD litters had abnormalities detected by free hand sectioning, and 29/177 in 12 of 22 HD litters had abnormalities detected upon skeletal exam. Results of study 182F/852 were similar to those of 11F/852 in that post-implantation loss was increased and fetal weight was slightly decreased at the HD which was teratogenic. The nature of the fetal abnormalities differed somewhat from the previous study. There was no increase in cardiac aberrations; rather, abnormal

liver lobation, reduced thyroid, unossified sternebrae, and other skeletal changes were observed. Many individual abnormalities were only slightly increased in incidence, but their occurrence suggests a widespread drug effect. On a mg/m^2 basis, the NOEL of 56 mg/kg for teratogenic effects provides a safety ratio of 7.6 when compared to the human MRDD of 60 mg. The pharmacokinetic data supplied (Cmax for non-pregnant rats administered 40 mg/kg, 2 hr plasma levels for pregnant rats administered 64, not 56, mg/kg and Css levels for humans given the 60 mg MRDD) are not sufficient to allow an exposure comparison.

Unlike in the rat Segment II studies, citalopram was not teratogenic in rabbit Segment II studies (0.8, 4.8, 16 mg/kg and 0.8, 3.2, and 12.8 mg/kg p.o.). Doses of 16 mg/kg produced at least 1 drug-related death, and in a preliminary segment of the study, 40 mg/kg dosing was associated with deaths in 7/8 animals; thus, dose selection was adequate. The incidence of minor anomalies was increased from 6% in controls to 14% at 16 mg/kg, but half of these findings were in fetuses from one doe in which the placenta had degenerated, which suggests that the anomalies were secondary to the placental degeneration. The NOEL for teratogenicity of 16 mg/kg provides a safety margin of 4.3, based on mg/m^2, relative to the human MRDD of 60 mg.

In the Segment III rat study (4.8, 12.8, 32 mg/kg p.o.; although the sponsor referred to several studies as Segment III, only study 200F/852 examined the reproductive performance of the F1 generation, and thereby meets the definition of a Segment III study), mammary papilla immaturity in HD dams likely caused the loss of 4/24 litters. A fifth HD litter was stillborn. Birth weight of HD pups was decreased and remained depressed throughout the study (70 days) in M and for 35 days in F. Incisor eruption was delayed in HD animals, but other developmental milestones were unaffected. Reproductive performance of the F1 generation was unimpaired, and there was no treatment effect on F2 fetal weight or external malformations. Based on litter loss and decreased F1 fetal weight, the NOEL is 12.8 mg/kg, providing a safety ratio of 1.7 on a mg/m^2 basis when compared to the human MRDD of 60 mg. In light of the effect on maternal mammary papilla that effectively limited dosing by decreasing pup viability, an additional study in which higher doses were administered on GD17-20 and pups were cross-fostered would have been valuable.

Genotoxicity

Citalopram was evaluated for genotoxicity using an Ames test, a chromosomal aberration test in Chinese hamster lung cells, an HPRT mutation test in mouse lymphoma cells, a chromosomal aberration test in human lymphocytes, an unscheduled DNA synthesis test, and two *in vivo* micronucleus tests. There were positive results in the first two tests and an equivocal result in the third test; the other tests were negative. In the Ames test, a reproducible, concentration-dependent increase in the number of TA98 revertants occurred under -S9 conditions. Also under -S9 conditions, the number of TA1537 revertants was increased 2 - 3-fold in two experiments; negative results were generated in experiments that did not use suitable concentration increments. When tested at appropriate concentrations, citalopram increased chromosomal aberrations in Chinese hamster lung cells in 2 of 3 -S9 experiments and 3 of 3 +S9 experiments. Unlike the results in hamster lung cells, an increase in chromosomal aberrations in human lymphocytes was not observed. Results of the HPRT mutation assay were positive under +S9 conditions at the greatest analyzable concentration in one experiment, only at intermediate concentrations in a second experiment, and not at all in a third experiment.

Carcinogenicity

Two carcinogenicity studies were conducted, one in NMRI mice and one in Wistar rats. In the 18 month mouse study, citalopram was not tumorigenic. Non-neoplastic pathology was limited to urinary bladder distension associated with other bladder and renal pathology in treated

males, liver necrosis and congestion in HD M, and cystic endometrial hyperplasia in LD and MD F. Plasma levels measured at study termination indicate that concentrations of citalopram and two demethylated metabolites increased with dose. The HD is acceptable as an MTD based on the convulsions that occurred in of HD M and F during the latter half of the study, the increased mortality observed in HD M, and the decrease in BW relative to control that occurred in HD M and F.

In the 2 year rat study, potentially treatment-related tumors were limited to a small number of small intestine carcinomas in LD and MD M, kidney carcinomas in MD F, mammary adenofibrosarcomas in MD F, and squamous papillomas in MD M. The overall incidence of small intestine carcinoma (8/200 treated animals versus 0/200 controls; historical control range of was determined by the Executive Carcinogenicity Assessment Committee to be suggestive of a treatment-related effect on a relatively rare tumor type. Absence of such findings in HD animals was discounted due to the markedly low BW (30% less than control) of HD M and F. Non-neoplastic pathology included increased incidences of lymph node hyperplasia in MD F, and macrophage accumulation in the lungs of MD M and HD M and F, a finding that also occurred in the 1 year general toxicology study. An increased proportion of HD M had small, flaccid testes that exhibited focal calcification, and an increased proportion of HD F had ovarian follicular cysts. There was an increased incidence of retinal degeneration in HD M and F, beyond the high background control incidence. Other ocular changes included an increase in lens (male) and corneal opacities (female). The retinal degeneration is proposed to result from increased penetration of light into the eye subsequent to treatment-induced mydriasis. Although mydriasis was not noted in this study, it was observed in an albino rat teratology study and in dog studies. Furthermore, because eyes of albino rats lack melanin, retinal damage is not likely related to the demonstrated accumulation of citalopram in the uveal tract of pigmented rodents. As in the mouse study, plasma levels measured at study termination indicate that concentrations of citalopram and two demethylated metabolites increased with dose.

Reduced BW may have contributed to the increased survival of HD rats. The modestly decreased BW experienced by MD animals suggest that this dose, or one between the MD and HD, should have been used as a HD. Although the study indicates limited carcinogenic potential for citalopram, interpretation of the results is complicated by the fact that citalopram was shown to degrade in the feed to the N-oxide. Thus animals may have been underdosed with citalopram, and the reduced BW used to define an MTD may have resulted from exposure to the N-oxide, rather than citalopram. The N-oxide has been identified as a minor metabolite in both rat and human urine, but not in plasma; N-oxide in urine accounts for 0.6% of a 40 mg dose in humans.

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DRAFT LABELING

RECOMMENDATION

The NDA is approvable with respect to the pharmacology/toxicology portion pending labeling revision (see labeling recommendations made in the Summary and Evaluation).

/S/

Robin A. Huff, Ph.D.

cc: NDA20822
HFD-120
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