CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20822

PHARMACOLOGY REVIEW(S)

REVIEW OF NDA SUPPLEMENT FOR CITALOPRAM, NDA 20-822

Letter Date: 5/22/98
Stamp Date: 5/26/98
Review Date: 6/2/98
Sponsor: Forest Laboratories, Inc.
Reviewer: Robin Huff
Drug: citalopram
Mechanism: selective serotonin reuptake inhibitor
Indication: depression

The sponsor has filed a class I resubmission in response to an approvable letter issued on May 12, 1998. A range-finding fertility study in rats was included in the submission and is reviewed below. In addition, numerous pharmacology and toxicology labeling changes have been requested, each of which is addressed individually.

I. Range-finding Fertility Study in Rats (CITTX05000) draft report

Sprague Dawley - 16/24 (M/F), 32, 48, 72 mg/kg base equivalents
8/s/gr

Dosing was initiated 70 and 15 days prior to mating for males and females, respectively. Dosing continued until males were killed and through day 7 of lactation for females. Clinical signs displayed by both males and females included a dosage-dependent increased incidence of salivation and perioral substance. In males, BW gain was decreased throughout dosing at 72 mg/kg/day, such that rats weighed 9% less than controls by the mating period and 15% less than controls by day 102. The BW gain decrement was paralleled by a 9% decrease in food consumption throughout treatment. In females, BW gain was decreased prior to mating and again during GD15-20 in all treatment groups, such that prior to mating BW at 24, 32, 48, and 72 mg/kg/day was 2, 3, 3, and 5%, respectively, less than control, and on GD20 BW at 24, 32, and 48 mg/kg/day was 3, 5, and 8% less than control, respectively (there were no pregnancies at 72 mg/kg/day). Food consumption prior to mating was decreased 11, 15, 15, and 25% at 24, 32, 48 and 72 mg/kg/day, respectively, but effects during gestation were minimal.

Mating was adversely affected by treatment, both in terms of percentage of matings and in the time delay prior to mating. In addition, copulatory plugs were smaller in all treated groups, and testes weight in the 72 mg/kg/day group was reduced 29%/17% (absolute/relative). The data are summarized in the table below and demonstrate that of the matings that occurred, a decreased percentage were successful at doses ≥32 mg/kg/day. In addition, gestation duration was increased from 23.0 to 24.0 days at 48 mg/kg/day, and was stated to be outside the historical control range.

Dose (mg/kg/day)	Number Mated M	F	Number Pregnant	Number Delivered
0	8	8	8	7
16/24	7	7	7	7
32	5 (of 7)	6	4	4
48	7	7	5	5
72	4 (of 7)	5	0	-

the number of animals mated was 8/s/gr except for males at 40 and 90 mg/kg/day (there was one accidental death in each of these groups)

Litter size and implantation sites per litter were decreased at 48 mg/kg/day, and pup survival was decreased at all doses as summarized in the table below. The decrease in pup survival reflects 2, 1, and 3 total litter losses by day 4 at 24, 32 and 48 mg/kg/day. Pup weights were decreased in all treated groups, and appeared to recover by day 7, but the number of litters surviving to day 7 was limited to 5, 3 and 1 at 24, 32, and 48 mg/kg/day, respectively.

Dose (mg/kg/day)	Litter Size (live pups)	Implantation Sites per Litter	Pup Survival (% on day 7)	Pup Weight (g) day 1	day 4	day 7
0	16.1	17.6	96.5	6.4	8.9	13.0
16/24	12.4	13.1	73.6	6.0	9.1	14.1
32	14.8	17.5	66.1	5.2	7.4	11.8
48	10.2	10.4	7.3	6.1	8.6#	14.7#

#n = 1 litter

times the MRHD."

III. Other Recommendations

/S/

Robin A. Huff, Ph.D.

cc: NDA20822B
HFD-120
/R. Huff
/G. Fitzgerald
/T. Laughren
/P.David

I concur with all recommendations
6/2/98
/S/

TO: Kathryn Bishburg, Pharm.D.
Associate Director
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Forest Laboratories

FROM: Todd Sahlroot, Ph.D.
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TOPIC: Needed carcinogenicity data analyses for NDA 20-822 citalopram

DATE: January 14, 1998

For statistical review of this application's preclinical carcinogenicity data, please provide the following:

(1) **Study Design**: Fully describe the design for each study including: strains of rodents, route of administration, time of interim and terminal kill, the number of animals used per dose group, and the type of control used (e.g., vehicle only).

(2) **Needed Statistical Analyses**:

Note: All analyses should be performed for each sex separately.

[A] **Survival**: Please provide trend tests, adjusted Cox and Kruskal-Wallis testing, all pairwise comparisons of all groups (with adjusted Cox and Kruskal-Wallis), and Kaplan Meier survival curves. See references (1), (2), and (3). Weigh doses by the actual dose levels used.

[B] **Tumor Analysis**: Use Peto's survival-adjusted trend tests appropriate for fatal, incidental and palpable tumors (reference (4)). Use the actual doses as weights. We suggest using fixed time intervals, e.g., weeks 0-52, 53-78, 79-91, 92-104, and terminal sacrifice. Perform **exact permutation trend tests** (e.g., StatXact software, reference (5)) and asymptotic tests when

combined fatal and incidental tumors fall in the same time interval or when the number of tumors is very large. Pairwise comparisons between high dose and control are optional. Statistical significance levels to be used are 0.025 for rare tumors and 0.005 for common tumors. If other levels are chosen (e.g., Westfall-Young), justification for the choice needs to be provided. Certain tumors should be also grouped and then analyzed. Please refer to reference (9).

[C] If a study shows no tumorigenic effect (for a given species and sex), please document the validity of the study (references (6), (7), and (8)) establishing that there were enough animals exposed for a sufficient length of time for late developing tumors to manifest and that the high dose represents a reasonable tumor challenge.

(3) For **guidance** we are providing the **following materials**:

[A] A representative statistical review of carcinogenicity studies which can be used as a model for presenting analytic results in a statistical report.

[B] Several internal SAS programs (still in the development stage) which can be used as a framework for your analytic approach.

[C] Diskette containing raw data for the rat and mouse studies.

(4) **Data**: In addition to the needed statistical report of analytic results, please return the animal data on diskette.

REFERENCES:

(1) Cox, D. (1972). Regression models and life tables, **Journal of Royal Stat. Soc. B**, 34, 187-200.
(2) Gehan, E. (1965). A generalized Wilcoxon test for comparing arbitrarily singly censored samples, **Biometrika**, 52, 203-223.
(3) Thomas, Breslow, and Gart (1977). Trend and homogeneity analyses of proportions and life table data, **Computers and Medical Research**, 10, 373-381.
(4) Peto et al. (1980). Guidelines for sample sensitive significance test for carcinogenic effects in long-term animal experiments, long term and short term screening assays for carcinogens: A critical appraisal, **International Agency for Research Against Cancer Monographs, Annex to Supplement**, WHO,

Geneva, 311-426.
(5) StatXact-3 for Windows (1996). Statistical Software for Exact Nonparametric Inference, Cytel Software Corporation, Cambridge, Massachusetts.
(6)Haseman (1984). Statistical Issues in the Design, Analysis and Interpretation of Animal Carcinogenicity Studies, **Environmental Health Perspectives**, Vol. 58, 385-392.
(7) Chu, Cueto, Ward. (1981). Factors in the evaluation of 200 National Cancer Institute carcinogen bioassays, **J. Of Toxicology and Environmental Health**, Vol. 8, 251-280.
(8) Bart, Chu, Tarone, (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity, **J. Of the National Cancer Institute, 62**, 957-974.
(9)McConnell, Solleveld, Swenberg, Boorman (1986). Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenisis Studies, **J.Of the National Cancer Institute, 76**, 283-289.
(10) Lin (1997). Formats and Specifications for Submission of Animal Carcinogenicity Study Data, OEB document.

In case of questions, the contact person is Ms. Roswitha Kelly, (301)827-1547.

cc:

HFD-024/Dr. DeGeorge
HFD-120/Drs. Fitzgerald, Huff
HFD-120/Mr. David, CSO
HFD-710/Drs. Sahlroot, Chi
HFD-710/Ms. Kelly
HFD-710/file

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Review of **NDA 20-822**

Drug: **Citalopram**, tablets

Sponsor: Forest Laboratories, Inc.
909 Third Avenue
New York, NY 10022-4731

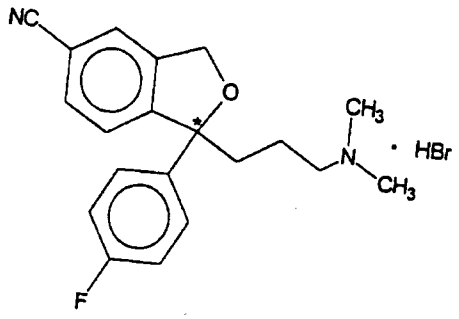
Review Date: February 13, 1998

Reviewer: Robin Huff

Class: selective serotonin reuptake inhibitor (SSRI)

Indication: depression

Structure:



Chemical Name: 1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, HBr

Molecular Formula: $C_{20}H_{21}FN_2O$ HBr

MW: 405.35

Related INDs/NDAs IND

Table of Contents

Note: Doses are reported as citalopram base, unless otherwise noted (base = 0.8 x HBr salt).

I. Pharmacology

Citalopram is a selective serotonin reuptake inhibitor (SSRI). The (+)enantiomer is the active moiety, and the (+)enantiomer of the monodesmethyl metabolite possesses 12 - 14% of the activity of the parent molecule. Citalopram inhibits uptake by binding to the high affinity imipramine site on the serotonin transporter. The *in vitro* selectivity for serotonin uptake inhibition (versus NE and DA) is ≥4-fold better than sertraline, paroxetine, fluvoxamine, fluoxetine, DCT, clomipramine, and amitriptyline. Binding and uptake experiments are summarized in the following sponsor-supplied tables.

Binding of Citalopram

Receptor	Ref.	System	Ligand	IC_{50} (μM)
5-HT_{1A}	10	Rat brain	^{3}H-5-HT	>100
5-HT_{2A}	10 11,12 13	Rat cortex Rat cortex Human cortex	^{3}H-spiroperidol ^{3}H-spiroperidol ^{3}H-ketanserin	8.76 9.2 9.5
DA D_1	11	Rat striatum	^{3}H-piflutixol	240
DA D_2	11 10 12	Rat striatum Rat striatum Rat striatum	^{3}H-haloperidol ^{3}H-spiroperidol ^{3}H-spiroperidol	19 61.0 34
α_1	10 11,13	Rat brain Rat brain	^{3}H-WB4101 ^{3}H-prazosin	3.65 1.6
α_2	10	Rat brain	^{3}H-PAC	54.5
Muscarinic	10 11 12	Rat brain Rat brain Rat brain	^{3}H-QNB ^{3}H-PrBCM ^{3}H-PrBCM	12.6 4.6 4.8
β	10	Rat brain	^{3}H-DHA	>100
GABA	11	Rat brain	^{3}H-GABA	740
Benzodiazepine	11	Rat cortex	^{3}H-flunitrazepam	>1,000
H_1	10	Rat brain	^{3}H-mepyramine	2.84

Binding of Citalopram and Desmethylcitalopram Enantiomers

IC_{50} Values in nM							
Receptor	Ligand	Citalopram (racemic)	Citalopram (+)	Citalopram (-)	Demethyl-Citalopram (racemic)	Demethyl-Citalopram (+)	Demethyl-Citalopram (-)
D_1	^{3}H-SCH23390	22,000	32,000	16,000	32,000	>10,000	>10,000
D_2	^{3}H-spiperone	33,000	>100,000	11,000	53,000	89,000	27,000
5-HT_{1A}	^{3}H-8-OH-DPAT	15,000	17,000	>100,000	41,000	31,000	>100,000
5-HT_2	^{3}H-ketanserin	3300	13,000	6300	8700	11,000	6700
α_1	^{3}H-prazosin	1600	5500	1600	1500	4300	650
α_2	^{3}H-idazoxan	18,000	20,000	12,000	23,000	39,000	16,000
β	^{3}H-dihydroalprenolol	>100,000	>100,000	>100,000	>100,000	>100,000	>100,000
H_1	^{3}H-mepyramine	350	2100	280	1700	4200	1000
Muscarinic	^{3}H-QNB	5600	3800	11,000	14,000	NT[2]	NT

[1] Data from Ref. 8
[2] NT = Not Tested

Serotonin Uptake Experiments

Test	System	Ref.	Effect
5-HT uptake	Cloned transporter	1	K_i= 6.1±1.0 nM compared to 33±1.0 for fluoxetine and 209±28 for imipramine.
5-HT uptake	Cloned transporter	2	K_i=10.1±2.6 nM compared to 51.1±1.4 for fluoxetine and 240±64 for desipramine.
5-HT uptake	Rat brain	3	Saturable and reversible binding with K_D=0.8 nM; binding correlated with synaptosomal serotonin uptake (r=0.97). Autoradiographic distribution of citalopram resembled distribution of serotonin.
5-HT uptake	Rat brain	4	Citalopram inhibited ^{3}H-imipramine binding and 5-HT uptake with IC_{50} values of 20 and 40 nM, respectively. Both high and low affinity binding for ^{3}H-imipramine were observed. Citalopram selectively inhibited binding at the high affinity site.
5-HT uptake	Human platelet	5	IC_{50}=25 nM for inhibiting binding by paroxetine and 2 nM for imipramine. The Hill coefficient for inhibiting binding to paroxetine was 1.08 and for imipramine was 0.55. Other SSRIs, but not tricyclic antidepressants, had similar Hill coefficients.
5-HT uptake	Rat brain	6	K_i=0.65 nM for citalopram inhibition of ^{3}H-paroxetine binding. K_i=14.2 nM for imipramine inhibition of paroxetine binding.
5-HT uptake	Rat cortex	7	Displaced ^{3}H-imipramine from serotonin uptake site with IC_{50}=38 nM, similar to fluoxetine (23 nM).
5-HT uptake	Rat brain synaptosomes	8,9	Citalopram and its (+) enantiomer inhibited 5-HT uptake with IC_{50} values of 1.8 and 1.5 nM, respectively while the IC_{50} was 250 nM for the (-) enantiomer. The IC_{50} values for the DCT [racemic (+), (-)] was 14, 9.9 and 65 nM, respectively.

[1] Refs. 1, 3-5, 7: the form of citalopram was not specified; Ref. 2: citalopram oxalate; Refs. 8,9: DCT-HCl; the enantiomers of citalopram and DCT were oxalate salts

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The primary pharmacological effects of citalopram were investigated in a variety of animal systems. In addition to characterizing the effect of citalopram on receptor and uptake systems, the sponsor evaluated the antidepressant activity of citalopram in multiple animal models. The results of these studies are summarized in the following sponsor-supplied table.

Test	Species	Route	Dose Range (mg/kg)[2]	Ref.	Effect (Lowest Dose Observed) (mg/kg)[1]
Uptake/Receptors					
	Rat	s.c.	ND[3]	17	Citalopram reversed H75/12-induced depletion of 5-HT in synaptosomes from rats given citalopram *in vivo* with an ED_{50} of 0.8 mg/kg. Of the citalopram metabolites, only the N-oxide metabolite showed any activity in the model (ED_{50}= 8.4 mg/kg). The ED_{50} for reversal of H77/77- induced NA depletion by citalopram and its didemethyl and N-oxide metabolites was >160 mg/kg. The ED_{50} for the demethyl metabolite was >80 mg/kg.
	Rat	s.c.	10	26	5-HT levels were unchanged 24 hours after a single injection of citalopram, while 5-HIAA was decreased. Reduced 5-HT turnover was observed which is expected when reuptake of 5-HT is inhibited.
	Rat	i.p.	0.5[4]	27	Increased 5-HT levels and decreased 5-HIAA levels in cerebellum, medulla and whole brain.
	Mouse	i.p.	20	28	5-HIAA decreased in cerebral hemispheres and brainstem 2 hours after citalopram administration.
	Rat	i.p.	0.25-40[4]	29	Decreased 5-HT synthesis rate observed, indicating feedback mechanism. ED_{75} after citalopram administration was 0.9 mg/kg.
	Rat	i.v.	0.05-5[5]	30	After a single dose of citalopram, inhibition of firing activity of 5-HT neurons was observed (ED_{50}=0.23±0.03 mg/kg). After 2 days' administration, marked reduction in firing activity was observed in nucleus raphe dorsalis; partial recovery after 7 days of treatment and total recovery after 14 days. Citalopram (20 mg/kg/day, i.p.) attenuated LSD-induced inhibition of nerve firing and enhanced the firing of hippocampal neurons in response to electrical stimulation after 14 days of treatment but not after a single dose. Enhanced effectiveness of 5-HT neurotransmission following 14 day citalopram treatment might be due to desensitization of the inhibitory 5-HT autoreceptor at the cell body level. Citalopram did not enhance increased firing of hippocampal neurons by iontophoretically-applied 5-HT.

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Test	Species	Route[1]	Dose Range (mg/kg)[2]	Ref.	Effect (Lowest Dose Observed) (mg/kg)[3]
Uptake/Receptors (Cont.)					
	Rat	Oral diet	10-40	31	Administration of citalopram for 2 weeks led to a marked and prolonged (up to 90% for at least 2 days after termination of drug treatment) inhibition of 5-HT uptake in platelet synaptosomes. *Ex vivo* citalopram inhibited 5-HT uptake in brain synaptosomes from animals given citalopram *in vivo* to the same extent as in control rats not treated with citalopram. No change in D_2 or β-adrenergic receptor binding was found in treated rats.
	Rat	i.p.	10[4]	32	Citalopram at 10 mg/kg/day for 20 days did not alter the number of 5-HT_2 cortical receptors.
	Rat	Oral	7.5-60[6]	33	Citalopram decreased blood concentrations of 5-HT to 33, 24, 7 and 3% of control at doses of 7.5, 15, 30 and 60 mg/kg. Blood concentrations were also decreased by the (+) and (-) enantiomers.
	Rat	i.p.	1,10	34	Citalopram increased extracellular 5-HT in hippocampus at 1 and 10 mg/kg, also by direct infusion; pretreatment with 10 mg/kg for 14 days did not affect the increase observed after acute administration.
	Rat	s.c.	5,10[5]	35	At both dose levels, treatment for 14 days enhanced the increase in extracellular 5-HT levels in dorsal hippocampus seen with infusion of citalopram (1 μM); this effect not observed in the frontal cortex. Decreases in 5-HT produced by infusion of the 5-HT_{1A} receptor agonists CP93,129 or RU24969, or systemic administration of citalopram (0.5 mg/kg, s.c.), were not different between treated and control groups in either brain site, indicating chronic administration of citalopram had no effect on nerve terminal or somatodendritic autoreceptor sensitivity.
	Rat	Oral	20	36	Citalopram was given to rats for 14 days and the brains of these animals were analyzed for NA and DA content. There were no changes in NA and DA.
	Rat	Oral diet	10-40	37	Rats were given citalopram for 13 days (40 mg/kg/day in the diet) and then studied for behavioral changes after amphetamine and 5-HT agonist challenge. Rats responded to d-amphetamine with an enhancement of induced hypermotility and stereotypy. There was a decreased behavioral responses to the 5-HT agonist 5-methoxy-N,N-dimethyltryptamine. These effects were not seen after acute dosing.

Test	Species	Route[1]	Dose Range (mg/kg)[2]	Ref.	Effect (Lowest Dose Observed) (mg/kg)[1]
Uptake/Receptors (Cont.)					
	Rat	Oral diet	40[5]	38	Citalopram treatment for 4 weeks had no effect on baseline or forskolin-stimulated (20 μM) cAMP levels in the dorsal hippocampus but decreased noradrenaline-induced (1 μM) enhancement of cAMP levels. Direct perfusion of the hippocampus with citalopram (10 μM) had no effect on cAMP levels.
	Rat	s.c.	2.5-20[4]	39	After 14 days treatment at 10 mg/kg, citalopram increased ^{125}I-iodocyanopindolol binding to β_1-adrenergic receptors by 10-20% in the somatosensory area of the frontal cortex, the caudate putamen, and the outer layers of the cingulate cortex. Fluoxetine at 2.5 mg/kg had same effect in frontal cortex and caudate putamen. Imipramine (15 mg/kg) decreased binding in these areas.
	Rat	i.p. s.c.	10[5]	40	After single i.p. dose, no change in GABA levels, GABA receptor binding or GABA uptake. After 18 days' s.c. exposure, $GABA_B$ binding in frontal cortex was increased 173%.
	Rat	i.p.	10[6]	41	Citalopram, imipramine and other tricyclics were given to rats for 3 and 6 weeks. α_1-adrenoceptors were upregulated and α_2-adrenoreceptor density decreased with all compounds.
	Rat	i.p.	10[5]	42	Rats were treated for up to 24 days with citalopram and other antidepressants. All drugs tested increased the density of α_1 adrenergic receptors without affecting affinity. Citalopram had no affect on α_2 receptor number.
	Rat	i.p.	10	43	Citalopram, tricyclic antidepressants and electroconvulsive shocks decreased the number of D_1 but not D_2 binding sites.
	Rat	p.o.	30[5]	44	Animals were given LiCl concurrently with citalopram, other SSRIs, or tricyclic antidepressants. Citalopram and other SSRIs had no effect on the NA response of the cAMP system in cortical slices, while tricyclic compounds reduced the response.
	Rat	i.p.	10	45	Citalopram administration for 4 weeks had no effect on the β-adrenoceptor system or the NA-sensitive cAMP response.

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Test	Species	Route	Dose Range (mg/kg)[2]	Ref.	Effect (Lowest Dose Observed) (mg/kg)[1]
Antidepressant Activity					
Forced Swimming	Mouse	s.c.	0.01-40	47	Significant reversal of immobility at all doses tested (0.01-40 mg/kg; 0.025-99 µmol/kg) as compared to imipramine which reversed immobility at ≥32 µmol/kg.
Forced Swimming	Rat	i.p.	16-64	46 48	Inactive at all doses. Other compounds of the class were inactive or active only at toxic doses.
Learned helplessness	Rat	i.p.	0.5 b.i.d.[5]	49	No effect on escape failure rate induced by inescapable shock after acute or subchronic administration prior to the session.
Learned helplessness	Rat	i.p.	1-2 b.i.d.[5]	50	Reversal of escape failure rate when administered 30 min after the learning sessions, but not before the sessions. Effect was the same at both doses.
Chronic mild stress	Rat	i.p.	10[5]	51	Animals subjected to mild stressors for 3 weeks prior to treatment and for 6 weeks after treatment. Citalopram significantly improved sucrose intake in stressed rats after 2 weeks of treatment; effect maintained through end of study.
Anxiolytic Activity					
Conditioned fear	Rat	s.c.	0.1-10	52 53	Decreased "freezing" response at 1-10 mg/kg, significantly ($p<0.05$) at 10 mg/kg (53% decrease from control). Did not alter locomotion or number of rearing events.
Conditioned fear	Rat	s.c.	0.1-10[5]	54	Significantly decreased "freezing" response at 1-10 mg/kg when locomotor activity was determined from 5-10 minutes after beginning of test.
Exploring	Mouse	s.c.	0.1-5	47	Increased time spent in light areas of box and increased crossing in the light area, statistically significant ($p<0.05$) at 0.25-5, respectively.
Exploring	Rat	s.c.	10^{-4}-0.1	47	No effect at 1/2 maximum intensity light. At maximum intensity, all parameters of exploration behavior increased at 1-10 µg/kg, but decreased at 100 µg/kg.
Footshock vocalization	Rat	s.c.	0.16-5	47	Inhibition of footshock-induced vocalization at all doses, statistically significant ($p<0.05$) at 0.32-2.6 mg/kg, with ED_{50}=0.3 mg/kg (1 µmol/kg). Paroxetine ED_{50} value was 0.86 µmol/kg while fluoxetine was >58 µmol/kg.

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II. Absorption, Distribution, Metabolism, and Excretion (ADME)

A. Absorption (pharmacokinetics)

Bioavailability of citalopram was approximately 50% in mouse, 50% in M rat, 80 - 90% in F rat, 30 - 93% in dog, 35% in monkey, and 40% in baboon. Bioavailability in humans was approximately 80%. After oral administration, Tmax ranged from in animals and was 4 hr in humans. The elimination half-life ranged from in animals, but was longer in humans, 37 hr. It was determined in monkeys that the $t_{1/2}$ for the didesmethyl metabolite substantially exceeded the $t_{1/2}$ for citalopram and the desmethyl metabolite, which is similar to what is observed in humans where the $t_{1/2}$ for the didesmethyl metabolite approximates 100 hr. Clearance of citalopram ranged in animals, but was greater in humans, 0.41 L/min/kg.

Cmax and AUC generally increased in a dose-proportional manner, and values tended to be greater in F than M animals. In dogs given citalopram orally for 8 days, each enantiomer of citalopram and the desmethyl metabolite accounted for approximately half of the respective Cmax and AUC; however, (+) didesmethyl citalopram accounted for approximately 3/4 of the didesmethyl metabolite, suggesting stereoselective removal of the second methyl group or stereoselective excretion of the didesmethyl metabolite.

B. Distribution

When pigmented male rats were given a single oral dose of radiolabeled citalopram, the highest concentrations of radiolabel were found in liver, adrenal, lung, uveal tract, spleen, kidney, pituitary, salivary gland, skin, blood, brain, and gonads (in descending order). Pigmented tissues (liver, eyes, skin) still contained radiolabel at 1 week, indicating persistent binding to melanin, as did gonads; t1/2's were 5.7, 16, and 41 days for gonads, eyes, and skin, respectively. When dogs were given 30 mg/kg p.o., killed after the onset of convulsions, and tissue extracts of lung, liver, kidney, heart and brain prepared, the highest level of citalopram was in the lungs, followed by liver and kidney, then by brain and heart. Metabolites were measurable in the lung and kidney. The volume of distribution was 25 L/kg in rats, 10 L/kg in dogs, and 12 L/kg in humans, reaffirming the extensive distribution seen in the studies described above.

Protein binding was 77, 71, 75, and 82% in mouse, rat, dog, and human plasma, respectively, and did not vary with concentration , tested for nonhuman species, 250 - 2500 nM tested for humans). Binding of the desmethyl metabolites was similar to binding of citalopram.

C. Metabolism

The primary metabolic pathway for citalopram involves two sequential demethylations to form the desmethyl and didesmethyl metabolites (DCT and DDCT). Subsequently the amide bond can be broken to form the propionic acid metabolite. DDCT is formed to a greater extent in dogs than in humans, primarily because first pass metabolism is greater in dogs. An alternative metabolism pathway involves oxidizing the nitrogen while leaving both methyl groups intact, resulting in the formation of the N-oxide. There is no mention of identification of N-oxide in plasma; however, it was found at low levels in some dog tissues and in rat, dog and human urine (rats and dogs were dosed for 3 months with 10 - 40 and 5 - 20 mg/kg/day, respectively). In humans at steady state, N-oxide in urine accounted for 0.6% of the 40 mg dose.

In vitro studies with cloned human P450 isozymes indicate that the initial demethylation of citalopram is carried out with high affinity by the 2D6 isozyme (K_m = 14 μM) and with lower

affinity by the 3A4 and 2C19 isozymes (K_m = 245 and 141 μM). The second demethylation is affected only by 2D6 (K_m = 10 μM). Rats that have P450 levels induced by two weeks of phenobarbital treatment, have a decreased $t_{1/2}$ for citalopram. In humans, a 40 mg dose of citalopram increased the AUC associated with a 40 mg dose of desipramine by 46%.

As mentioned above, 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.

D. Excretion

The majority of orally administered citalopram is excreted as citalopram, DCT and DDCT, in both animals and humans. Smaller amounts of proprionic acid, N-oxide, a tertiary amine with an open ring, and other unidentified metabolites are also excreted, with some metabolites being glucuronidated. In rats given a 20 mg/kg oral dose, 43% of the dose is excreted in the urine and 35% in the feces within 48 hr (total recovery was only ~80%), with most metabolites identified in both urine and feces. A study conducted in dogs indicates significant concentrations of citalopram and metabolites 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, 46% of the dose was excreted in urine (23% citalopram, 19% DCT, and 4% DDCT). Hepatic elimination is substantial in humans as hepatic insufficiency increased the $t_{1/2}$ from 37 to 83 hr.

III. Chronic Toxicology

A. Rats, 1 year oral dietary mix (685F) GLP, QA

Wistar rats - 32, 60, 120 mg/kg (batch 1031)
20/s/gr (C and HD: + 10/s/gr for 4 wk recovery; + 10/s/gr for 13 wk recovery)

Note: 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 unknown because analysis was delayed generally 3 - 6 weeks. 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, ~300 ppm LD diet samples from the carcinogenicity study that were analyzed 1 week after preparation contained 6% N-oxide, and samples analyzed 2 weeks after preparation contained 15% N-oxide, values that exceed the predicted degradation.

Mortality — A total of 12 animals died in the main and recovery studies, 7/80 C, 2/40 LD, 1/40 MD, and 2/80 HD; deaths were not treatment-related.

Clinical Signs — In the main study, seizure-like activity was observed once in MD and HD M, twice in LD F, 3 times in MD F, and 4 times in HD F. The episodes are described as 30 - 90 sec periods of "muscle spasms, acute violent twitching and jumping movements, rearing on hind limbs and making 'boxing' movements with their forelimbs, rapid respiration and gasping for air." Interestingly, in recovery groups, controls exhibited similar behavior, but the incidence was increased 3 - 4X in HD F. Incidences were 6, 3, 9, and 24 for 4 week recovery C M, HD M, C F, and HD F, respectively, and 6, 5, 8, and 32 for 13 week recovery C M, HD M, C F, and HD F, respectively. Because individual animals may have experienced more than one episode, it is not possible to determine the number of animals affected from the data provided. In addition to exhibiting 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 of the tail abnormality were dose-related.

Body Weight — BW gain was reduced in all treated groups. HD animals were affected as of week 2, MD M as of week 3, MD F as of week 5, LD M as of week 22, and LD F as of week 9. Week 52 BW was 36, 24, 19, 16, 6, and 7% less than control for HD M, HD F, MD M, MD F, LD M, and LD F, respectively. During recovery, BW gain rebounded in HD animals; the BW differential was reduced by 19 and 25% in M and F in four weeks. However, even after 13 weeks, BW was still 20 and 27% less than control in HD M and F, respectively. (The 27% difference in BW reported for recovery HD F is not an increase beyond the 24% reported in the main study; in recovery group HD F, BW was 30% less than control at the end of treatment.) Food consumption paralleled changes in BW.

Eyes — Ophthalmologic examinations following mydriasis were performed on control and HD animals during weeks 24 and 50. There were no treatment-related effects.

Hematology — Parameters were measured in 10/s/gr at 4, 13, 26, and 50 weeks. At 4 weeks the only notable change was a 7% increase in MCHC in HD M. This increase

persisted at 13 weeks, and increases were also detected in LD and MD M. At 26 and 50 weeks effects on MCHC were minimal. RBC's were decreased 4, 12, and 6% during week 13 in LD, MD, and HD F, respectively, yet at 26 weeks there was a 7% increase in HD F. At 50 weeks RBC's were decreased 8% in HD M; the decrease diminished to 4% after 3 and 12 weeks of recovery. Other changes that occurred in HD M at 50 weeks included a 10% decrease in Hb and a 13% decrease in PCV; both decreases steadily diminished during recovery, reaching 3% for Hb and 5% for PCV by 12 weeks. HD M also experienced a 65% increase in WBC and the differential count was redistributed with a 44% increase in neutrophils and a 17% decrease in lymphocytes. HD F experienced a similar redistribution, but only a 26% increase in WBC. The redistribution diminished during recovery, but was still detectable (23% increase in neutrophils/11% decrease in lymphocytes) in HD M after 12 weeks, at which point WBC's were increased 33% in HD M and 23% in HD F.

Lymphocytes were vacuolated in all HD animals at week 50; of lymphocytes were affected in M and of lymphocytes were affected in F. Also at week 50, 2/10 MD M and 2/10 MD F displayed occasional vacuolation. Varying degrees of vacuolation were detectable in HD animals at week 26. There was no evidence of vacuolation in 3 or 12 week recovery animals.

Clinical Chem

Parameters were measured in 10/s/gr at 4, 13, 26, and 50 weeks. AP was increased 40% in HD F at 50 weeks, but not during recovery. ALT was increased at week 13 by 31, 77, 40, and 64% in LD M, MD M, HD M, and HD F, respectively; increases were generally the result of 2 - 3 fold increases in some animals. AST was slightly increased in the same groups at 13 weeks, but individual values were not greatly increased over the highest control value, except for 2 HD F with increases of 51 and 86%. At 26 weeks, 2 MD M had ALT levels 1.7 and 4.3X the highest control value, accompanied by increases in AST. Two HD F had ALT levels that were 1.4 and 2.6X the highest control value. At 50 weeks, 1 HD M had an ALT level 5X and an AST level 2X the highest control. One HD F had an ALT level 2.5X and an AST level 1.4X the highest control. After 3 weeks of recovery, ALT and AST levels were increased 75 and 30%, respectively in HD M, with many animals contributing to the increase. At 12 weeks after recovery, there was 1 HD M with ALT and AST levels 4.5 and 3.3X the highest control; it is not possible to assess whether this indicates persistence of elevated ALT and AST, because data were collected from recovery group animals only after the recovery period.

Total protein was decreased in HD M and F at weeks 13 and 26, and 4% in 3 week recovery HD F. Albumin was decreased 15% in HD M at week 13, 20% at week 26, and 10% at week 50; decreases were in HD F at weeks 26 and 50. Although there was no decrease in 3 week recovery HD M, albumin was decreased 11% in 13 week recovery HD M; albumin was decreased 6% in 3 week recovery HD F. BUN was decreased in HD M and F by 16 and 9% at week 4, 17 and 23% at week 13, 9 and 14% at week 26, and 5 (ns) and 11% at week 50. There was no decrease in BUN in the recovery groups. At week 50, K^+ was increased in HD M and F, but individual values were within the normal range.

Triglycerides, cholesterol, and HDL, not measured at previous time points, were decreased [illegible] in HD M at 50 weeks and remained decreased throughout recovery. Interestingly, similar decreases were not seen in HD F at 50 weeks, but were seen in 3 week recovery HD F; the decrease in triglycerides was also present in 13 week recovery HD F. Lipid analysis of male liver samples (10/gr) revealed a dose-related increase in total lipids (37, 43, and 58% in LD, MD, and HD, respectively), a dose-related increase in cholesterol (10, 22, and 25% in LD, MD, and HD, respectively), and a doubling of triglycerides in all dose groups. After 4 weeks of recovery, increases had abated, and by 13 weeks individual values were within the normal range. The sponsor reports decreases after 13 weeks of recovery, but the reported decreases are due to 1 - 2 atypically high control values.

Urinalysis — Parameters were measured in 10/s/gr at 4, 13, 26, and 50 weeks. There were no treatment-related effects.

Organ Weights — The absolute weights of most major organs were decreased in HD animals, but organ weights were increased relative to BW, reflecting the decrease in BW. The increase in relative adrenal weight in HD M exceeded the increases in other organs; the increase was 72%, but was absent after 13 weeks of recovery. The relative increases in some organs persisted during recovery, reflecting the incomplete BW recovery by HD animals. The sponsor erroneously reports both an absolute and relative increase in spleen weight in HD M. The absolute HD mean was miscalculated; absolute weight was decreased, not increased.

Pathology — The corkscrew tail abnormality, described as a clinical sign, occurred in all HD M, approximately half of the HD F and MD M, and in few LD M and MD F. Tissues from control and HD animals were histopathologically examined. Liver, kidney, lung, thymus, and testes were examined in males of all dose groups and liver, lung, mammary gland, and uterus were examined in females of all dose groups. Tissues from recovery animals were examined macroscopically only, except for M livers sections which were stained with ORO.

Nearly all treated M displayed vacuolated hepatocytes and ORO-positive fat in the liver. Although ORO-positive fat was also observed in 9/20 controls, staining was more marked in treated animals. The increased incidence of ORO-positive fat persisted through 4 weeks of recovery; by 13 weeks the control incidence and severity had increased to rival that of treated animals. Five HD F (v. 0 controls) exhibited parenchymal inflammatory cell infiltration in the liver. Interstitial inflammatory cell infiltration occurred in the kidneys of 0 C, 2 LD, 3 MD, and 8 HD M; 2 HD M also displayed perivascular inflammatory cell infiltration. There was perivascular accumulation of lymphocytes in the lungs of 0 C, 6 LD, 6 MD, and 4 HD M, and the incidence and severity of macrophage accumulation was increased in all treated M and F groups. Macroscopically, there was a dose-related increase in white areas in the lungs of M, and multiple white nodules were detected in 4 HD M; the former finding persisted throughout recovery. The incidence of involution in the thymus was increased from 7 controls, to 10, 12, and 13 LD, MD, and HD M, respectively. Testicular tubular atrophy occurred in 10 MD and 15 HD M, compared to 1 control, and the severity was marked in 1 MD and 3 HD. There was tubular calcification in 13 HD M (versus 1 control) and spermatozoa in the epididymal ducts were reduced or absent in 5 HD M (versus 1 control).

B. Dogs, 1 year oral capsule (678F, 13/852, 14/852, 850/65, 17F/852, 349/67) GLP, QA

Beagle dogs - 1, 3, 8 mg/kg (batch 1002)
5/s/gr

Dr. Barry Rosloff's June 25, 1985, review of this study is excerpted below.

Beagles were treated with 0, 1, 3, or 8 mg/kg/day, in capsules (5 M + 5 F per group). Five deaths occurred at HD (2 M + 3 F), during weeks 17, 18, 27, 27, and 31, respectively. Deaths occurred within 2-3 hr post dosing with the first 4 dogs; the 5th was found dead 25 hours after the last dose, after having been last seen alive 9 hours post-dosing. (In addition, a control F and an MD M were sacrificed weeks 51 and 12, respectively; this was said to be on humane grounds to alleviate the discomfort caused by a sepsis in the cervical region which was not considered drug-related.)

The 5 deaths at HD were sudden and unexplained in that prior to death they were in generally good condition and had shown no consistent adverse effects on EKG or lab tests. Gross and histopathologic exam showed no cause of death. Two of the dogs died unobserved. Two penmates were observed trembling, collapsing and dying while completing their meals (2-3 hr post-dose, week 27). The fifth dog was found dead after a dog fight with a penmate; there were no bite wounds on the body.

There were few drug-related changes in this study, and of those seen none were clearly specific to the dogs that died (except for changes which were considered secondary to death, i.e. contracted hearts, ORO-negative vacuolation to hepatocytes, vascular congestion, and increased blood electrolytes in terminal samples). There were no clearly drug-related effects on bodyweight, food consumption, hematology, blood chemistry, urinalysis, or gross- or histopathological exam. Probable drug effects in this study included:

1) At MD, mydriasis (not seen after week 15) and salivation In F.

2) At HD, mydriasis accompanied by partial or no accommodation to light (not seen after week 15), and, in F only, salivation.

3) QT interval was slightly increased at HD, generally about 10% above controls. (The dogs which subsequently died tended to have higher values than survivors at HD; however, there was some overlap in values and it cannot be concluded that this effect was greater in the dogs which died). (EKG was performed prior to dosing weeks 6, 12, 25, 38, and 51).

4) Thymus weight increased at HD. (Values in decedents similar to those in survivors.)

It was stated that the animals in this study had been (inadvertently) subjected to sudden, loud noises during the study. It was thought that these noises may have triggered "fatal stress reactions" in the high dose dogs. To test this, an acoustic stress test was performed in surviving HD dogs prior to termination. There were no significant differences between control and treated dogs regarding behavioral or rectal temperature response to a loud noise.

Plasma levels of citalopram and its demethylated metabolites were determined in this study. At HD, levels in the dogs which later died (sampled weeks 4 and 26) were similar to those in survivors. Postmortem plasma levels were also determined in 4 of the 5 dogs that died; 1 or 2 of these had levels of citalopram and/or metabolites which were greater than those determined in

surviving dogs during the latter part of the study; however, since decedents and survivors were not sampled at the same time, and since the effects of postmortem changes on citalopram distribution, metabolism, assay procedure, etc., are not known, these levels are not directly comparable and it cannot be concluded that these 1 or 2 decedents were actually exposed to more drug than were the survivors. It should also be noted that while plasma levels of C and metabolites in HD dogs (both in survivors and decedents) tended to remain the same or be slightly increased between weeks 4 and 26, levels at later times (in survivors and in the 1 decedent who lived long enough for a subsequent *in vivo* sampling) were considerably greater

Evaluation

The relatively high vulnerability of the dog to the toxic effects of citalopram (C) has been previously noted (Preliminary Safety Review of 7-28-83; Original Summary of 9-19-83). An acute dose of 20 mg/kg p.o. produced mydriasis, restlessness, anxiety, tachycardia and highly labile heart rate, convulsions, and death. In a 3 month toxicity study, 10 mg/kg/day p.o. produced similar signs, and 4/7 deaths by week 7. (These deaths occurred weeks 3, 4, 7, and 7, resp; they were not observed but were assumed to be associated with convulsions. No deaths occurred at 5 mg/kg/day.) In a 6 month toxicity study, on the other hand, no deaths were seen up to 8 mg/kg/day p.o. (Plasma levels of C were measured in this 6 month study and compared with those obtained in the presently reviewed 1 year study; it was found that levels in the 1 year study were somewhat lower. Thus a greater plasma level does not explain the fact that deaths were seen in the 1 year study, but not at the same dose in the 6 month study. Also, the lower plasma level associated with lethality in the 1 year study necessitates a downward revision of the previous estimate of 6000 nM as a convulsive/lethal threshold in dogs, possibly to as low as

None of the dog toxicity studies which have been performed provides an explanation for the sudden deaths. There have been no pronounced drug effects on the usual toxicological parameters in these studies. In acute studies, toxic doses presumably caused death from convulsions; however, in the subacute studies, while both convulsions and deaths were seen at 20 mg/kg, no convulsions were observed with lower lethal doses (although they might have occurred unnoticed).

Since most drugs of this class are known to produce serious cardiac effects at higher doses, such effects should be considered as a possible cause of C-induced mortality. However, EKG was monitored in the 3, 6, and 12 month studies, and no pronounced effects were seen. In the 6 month study there was slight tachycardia at 3 and 8 mg/kg. In the 1 year study there was a 10% increase in QT interval at 8 mg/kg although this was not clearly associated specifically with the dogs that died. (In the 6 month study, it was not stated at what time relative to dosing the EKG was measured. In the 1 year study, EKG was measured prior to dosing [i.e., 24 hours after the previous dose], and thus any acute effects of the drug would have been missed). In acute cardiovascular studies in dogs, oral doses of ≥5 mg/kg caused tachycardia (in contrast to other species where decreased heart rate or no change was seen) and labile heart rate. Blood pressure responses at these doses were variable but increases were usually seen. No EKG abnormalities were seen up to 10 mg/kg p.o. (although the number of dogs studied was small); change in T wave polarity was noted at 20 mg/kg p.o. An increase in pulmonary artery pressure of was seen at 10 mg/kg p.o.. Cardiovascular studies in cats and rabbits (review of 9-19-83) suggested that C had a higher safety margin than several reference antidepressants; however, the fact that apparently not all of the compounds were studied in a single experiment, and the lack of adequate dose-response data, limits the confidence in this conclusion. In summary, there is no evidence or reason to suppose that the sudden deaths in the subacute dog studies were due to cardiovascular problems, although it should be noted that cardiovascular function was not assessed in these studies at or near the time of death.

Mice and rats can tolerate much higher doses of C than do dogs. Acute oral LD50 was about 1000 mg/kg in these species. Subacute oral doses of up to 200 mg/kg/day were generally well-tolerated by rats. At least part of the reason for the greater susceptibility of dogs is the much higher plasma levels achieved at a given dose. (There is no qualitative difference in metabolic pattern across species; rat and dog have more extensive demethylation than do mouse and human). The sponsor states that over 1300 human patients have been treated with C "without the occurrence of any drug-related deaths."

It should be noted that 2 other putative antidepressants recently reviewed by me were poorly tolerated by dogs, with convulsions and deaths seen at relatively low doses. (Fluoxetine, and fluvoxamine. . (In a 1 year dog study with fluoxetine, "unexplained" deaths occurred well into the study, however, they were not "sudden" in that the dogs were in visibly poor condition prior to death). Both of these drugs as well as C are considered to be "5-HT-specific" antidepressants (i.e., they block the neuronal re-uptake of 5-HT much more potently than that of norepinephrine). The present sponsor has suggested that C-induced convulsions in dogs were mediated by serotonergic hyperactivity, although this was based on limited data.

In summary, C has been shown to be poorly tolerated by dogs, with several sudden and unexplained deaths occurring well into subacute studies. The fact that dogs are particularly susceptible *per se* is not troubling, since species differences in sensitivity to drugs are common, and a high dose for a toxicity study is purposely chosen as one which produces adverse effects. However, the fact that these deaths were "sudden" (i.e., the dogs did not overtly appear to be in poor health) and that no causes for these deaths were established represents a gap in our knowledge of the toxicological profile of this drug, and does not suggest any useful monitoring in humans which may indicate a potential problem. That the deaths were due to convulsions or cardiac effects is a likely possibility, but one for which no direct evidence exists. Nor is there any indication whether the deaths were due to a cumulative process as opposed to an acute toxic effect occurring during chronic dosing. A toxicity study in monkeys would help to determine the species specificity of these findings.

IV. **Cardiovascular Toxicology** - special study

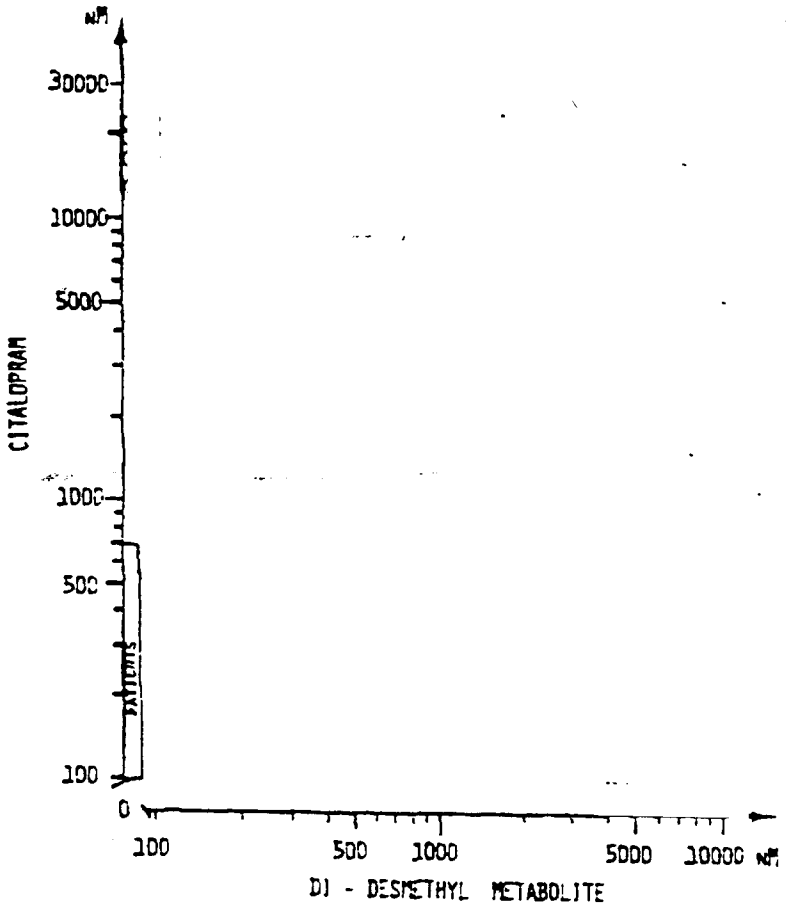
In an effort to further investigate the unexplained, sudden deaths that occurred in HD dogs during the 1 year study, cardiovascular function was monitored in dogs given acute, intravenous doses of citalopram and its didesmethyl metabolite. Dr Barry Rosloff's April 14, 1989, review of this study is excerpted below.

Although citalopram was already known to be poorly tolerated by dogs, the occurrence of sudden, unexplained deaths well into a 1 year oral toxicity study (deaths seen weeks 17-31) was troubling since prior to the deaths these dogs appeared to be in good health. Two possible causes of the deaths which were considered were convulsions and cardiac arrhythmias. Although convulsions were not observed in the 1 year dog study, convulsions associated with deaths were seen in previous acute studies in dogs. Cardiovascular effects were considered in view of the effects of related antidepressants, although acute studies suggested that citalopram had a higher cardiovascular safety margin than the reference drugs, and EKG effects in the 1 year study were limited to slight Q-T prolongation (although note that EKG in this study was measured 24 hours after the daily dose, thus missing any possible acute effects).

To help determine the possible cause of the sudden deaths in dogs, a study was performed) in which intravenous citalopram (CT), its didesmethyl metabolite (DDCT), and a combination of CT and DDCT were given acutely to Beagle dogs, and cardiovascular function measured. The dosages were as follows:

1. Saline control (N=3)
2. CT (10 mg/kg/hr) (N=4)
3. DDCT (2.5 mg/kg/hr) (N=4)
4. CT (10 mg/kg/hr) + DDCT (2.5 mg/kg/hr) (N=9)

Dogs in groups 2 and 4 received occasional doses of diazepam to try to decrease convulsions, which were seen only in these groups. (Some diazepam-only injections were given prior to the study as a control). A variety of parameters showed no clear drug effects, including heart rate, blood pressure, and cardiac histopathology. The primary EKG effect noted was a prolongation of the QT interval, which was pronounced in groups 3 and 4, suggesting this effect was primarily due to the DDCT. Ventricular arrhythmias were seen in groups 2 and 4; these were thought to occur secondary to the excessive (CT-induced) CNS stimulation seen in these groups. In addition, fatal ventricular arrhythmias were seen in group 4 (5/9 deaths), leading to the conclusion that deaths from the combination resulted from an interaction between the QT interval-prolonging effects of DDCT and the CNS effects (with resultant centrally-mediated ventricular arrhythmias) of CT. Plasma levels obtained in these studies are shown in the figure below; also shown are plasma levels from the 1 year dog study and from human patients.



Plasma levels of citalopram and di-desmethyl metabolite in the present, acute experiments (round symbols) and previous 12-month study (triangles). Open symbols denote surviving animals, closed symbols dogs that died. Individual data are given for dogs in acute experiments (highest values recorded) and for dogs in the top dose group of the chronic study (samples taken about 2 hours after the final dose) (Fredricson Overø 1984a, 1984b) Group mean data (2 hours after dosing, average of week 29-52) are given for intermediate and low dose groups in the 12-month study (Fredricson Overø 1984b). Also included in the lower left corner is the range of citalopram plasma levels in patients treated with the most common dose of 40 mg daily (Fredricson Overø and Sánchez 1985). Di-desmethyl metabolite levels above 90 nM have never been observed in man (Fredricson Overø 1986).

It can be seen that plasma levels of both CT and DDCT must be above certain thresholds for lethality to occur; when only 1 of these compounds was above threshold (e.g., groups 2 and 3) deaths did not occur. It can also be seen that plasma levels of CT and DDCT associated with lethality in the 1 year study were similar to those associated with lethality in the acute i.v. study, suggesting that the deaths in the former were due to the type of interactive mechanism discussed

above. (The levels of CT associated with lethality in the i.v study appear to be somewhat greater than those in the 1 year study, possibly because the former used diazepam to counteract CNS toxicity). (It should also be noted that the reason for the substantial plasma level of DDCT seen in the 1 year study, in contrast to the relatively small amounts seen after acute i.v. or p.o. dosing with CT, is likely due to the much longer $t_{1/2}$ of DDCT [1-2 days] and its subsequent accumulation with repeated dosing. It fits the sponsor's "interaction" hypothesis that the deaths seen in dogs after acute dosing with CT are due to primary CNS [as opposed to cardiac] effects, since little DDCT is formed. [See Boeck et al., Acta Pharmacol. et Toxicol. 50:169-174, 1982].) It can also be seen in the above figure that plasma levels of CT and DDCT in humans receiving 40 mg/day are well below those associated with lethality in dogs. (Another point made by the sponsor is that in addition to lower absolute levels of CT and DDCT, humans, by virtue of a smaller first pass effect, have a much greater CT/DDCT ratio than dogs, thus making humans less susceptible to the CT/DDCT interaction described above.)

Evaluation

The acute i.v. study described above represents an advance in our knowledge of citalopram (CT) in that it suggests possible mechanisms for the lethality of CT and demonstrates some toxicological differences between CT and one of its major metabolites didesmethylcitalopram (DDCT) (e.g., the latter more potently prolongs the QT interval, which may cause fatal arrhythmias in combination with the CNS effects [and consequent ventricular arrhythmias] of the parent compound). However, there are several problems in concluding that the proposed mechanism was responsible for the sudden deaths in the 1 year dog study:

1. It is not clear why a basically acute effect would not occur until 17-31 weeks of dosing. (If the $t_{1/2}$ of DDCT is 1-2 days, steady state levels would have been reached by 1-2 weeks.) In addition, several other antidepressants have similar or more pronounced adverse effects on cardiac conduction but (to my knowledge) have not caused sudden deaths with chronic dosing. It might be argued that the chronically CT-treated dogs had arrhythmias all along and that the deaths were triggered by stress, e.g., 1 of the 5 deaths was seen after the dog was observed fighting with a penmate. (As noted in my review of 6/25/85, the sponsor had considered that the stress may have been due to sudden loud noises which occurred during the study; however, an acoustic stress test in surviving dogs showed no effect.)

2. Although plasma levels of CT and DDCT associated with lethality were similar in the 1 year p.o. and acute i.v. studies, the rate of rise of levels in the i.v. study was likely much greater than in the p.o. study (where absorption and hepatic metabolism primarily control the rate of rise of CT and DDCT, resp.). The rapid rate of rise of plasma levels in the i.v. studies may have played a large part in the toxic effects observed.

3.The role of the mono-desmethyl metabolite (desmethylcitalopram, or DCT) was not taken into account. (The sponsor stated that "early acute studies in the dog" indicated that DCT did not play a role, but the reference cited [Boeck et al., ibid.] did not directly study the effect of DCT and concluded a contributory effect was possible). Levels of DCT were not measured in the acute i.v. study reviewed above. In a study in which dogs were given 20 mg/kg CT p.o. acutely, peak plasma levels of CT and DCT were and 2000 nM, respectively; levels of DCT declined more slowly than those of CT so that at later times levels of DCT were greater than those of CT (Boeck, ibid.). In this study, as well as in the above reviewed i.v. study, little or no DDCT is found in plasma after acute dosing with CT in dogs. The situation is different with chronic dosing, since the metabolites have longer half-lives than the parent compound (DDCT > DCT > CT). In the 1 year dog toxicity study, at 2 hours post-dose (8 mg/kg), levels of DCT were slightly less than those of DDCT; levels of CT were . At 24 hours post-dose, levels of DCT

and DDCT were greater than those of CT, respectively. In humans, the role of DCT relative to that of DDCT is likely to be greater than in dog: in patients receiving therapeutic doses of CT, steady state plasma levels of DDCT are usually below the limit of detection (i.e, levels at least 10x less than those of CT) whereas DCT levels are those of CT.

It is thus concluded that the cause of the sudden deaths in the 1 year dog toxicity study has not been established. While it is probable that the acute lethality of CT is due to a combination of CNS and cardiac effects, it is not clear why the lethality in the 1 year study did not occur earlier in the study if it were due to such an acute effect. On the other hand, a more insidious, chronic, cumulative toxic effect, e.g. a direct toxic effect on the heart demonstrable by histopathology, was not seen. The possibility may be considered that the deaths were due to some nonspecific factor (e.g., acute stress, or possibly a physiological state resulting in higher than usual plasma levels) superimposed on a near-lethal dose of CT. Oral doses not much greater than the dose causing the deaths in the 1 year dog study (8 mg/kg) are known to cause deaths associated with convulsions and/or cardiovascular changes in dogs. It is possible that convulsions occurred in the 1 year study but were unobserved; it is also possible that severe cardiac arrhythmias occurred but were undetected since EKGs were not measured until 24 hours post dosing (at which time a slight Q-T prolongation was noted).

The question of the relevance of the sudden deaths in dogs to humans must be addressed. As discussed above, the sponsor's argument regarding the role of the metabolite DDCT, which is not formed to a large extent in humans, is not convincing. It is noted that the plasma levels of CT, DCT, and DDCT are many fold lower in humans than in dogs; however species differences in sensitivity to a given level must be considered (e.g., no sudden deaths seen in rats despite plasma levels at least as high as those in dogs). The fact that no grossly observable signs were seen in the dogs which died is troubling (although note that unobserved convulsions may have occurred) since one would like to see warning signs in humans prior to the development of serious toxicity. The dog data suggest the utility of careful EKG monitoring, with possible Q-T prolongation as a warning sign. It is noted here that as part of the present submission, the sponsor has reviewed previous human experience with citalopram and has concluded that no drug-related deaths or serious cardiovascular effects have occurred; review of this data is outside the scope of the present review.