Lu 26-054-O and Lu 10-171-B Bridging Toxicity Study of Lu 26-054-O and Citalopram in Rats

Study No: 99358
Amendment #, Vol #, and page #: Vol 21 / Page 5-02867
Conducting laboratory and location: [redacted]
Date of study initiation: June 5, 2000
GLP compliance: Yes
QA- Report Yes (X) No ()
Methods:
Dosing:

- species/strain: Rat, Han Wistar (Crl: Han Wist (Glx.BRI) Br strain)
- #/sex/group or time point: see below
- age: 8-9 Weeks
- weight:
- satellite groups used for toxicokinetics or recovery: see below
- dosage groups in administered units: Duration of exposure was 60 days

Main Study (Heart Pathology)

Group	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	
			Males	Females
1	Vehicle	0	1-40	121-160
2	Lu 26-054-O	80	41-50	161-170
3	Lu 26-054-O	120/100[a]	51-70	171-190
4	Lu 10-171-B	80	71-80	191-200
5	Lu 10-171-B	120	81-100	201-220
6	Lu 10-171-B	160/100[b]	101-120	221-240

Satellite Study (Toxicokinetics)

Group	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	
			Males	Females
7	Lu 26-054-O	80	241-300	541-600
8	Lu 26-054-O	120/100[a]	301-360	601-660
9	Lu 10-171-B	80	361-420	661-720
10	Lu 10-171-B	120	421-480	721-780
11	Lu 10-171-B	160/100[b]	481-540	781-840

a = Dose level was reduced from 120 mg to 100 mg Lu 26-054-O.kg^{-1}.day^{-1} on Day 27 (Main study)/Day 22 (Satellite study)
b = Dose level was reduced from 160 mg to 100 mg Lu 10-171-B.kg^{-1}.day^{-1} on Day 9 (Main study)/Day 8 (Satellite study)

Figure 24, from page 19 of Study 99358

route, form, volume, and infusion rate:
Drug, lot#, radiolabel, and % purity: 006B, [redacted]
Formulation/vehicle: 0.9% Sodium Chloride
Observations and times:

- Clinical signs: 2X/day
- Body weights: 1X/week
- Food consumption: Not done
- Ophthalmoscopy: Not done
- EKG: Not done
- Hematology: Not done

- Clinical chemistry: Not done
- Urinalysis: Not done
- Organ weights: Day 60
- Gross pathology: Day 60
- Organs weighed: Heart only
- Histopathology: Heart only
- Toxicokinetics: Days 1, 8, 60; 0, 0.5, 1, 1.5, 3, 6, 9, 12, 24 hours post-dose
- Other:

Results:

- Clinical signs:

Group	Treatment ($mg.kg^{-1}.day^{-1}$)	Incidence of Decedent Animals	
		Males	Females
1	Vehicle (0)	0	0
2	Lu 26-054-O (80)	2	0
3	Lu 26-054-O (120/100)[a]	7	4
4	Lu 10-171-B (80)	0	0
5	Lu 10-171-B (120)	1	3
6	Lu 10-171-B (160/100)[b]	5	3

a = dose level reduced on Day 27
b = dose level reduced on Day 9

Figure 25, from page 28 of Report 99358

Clinical signs in premature decedents

Males

Observation/Finding	Group/Dose Level (mg Lu 26-054/ Lu 10-171.$kg^{-1}.day^{-1}$)					
	1 (0)	2 (80)	3 (120/100)	4 (80)	5 (120)	6 (160/100)
Number of Animals	0	2	7	0	1	5
Staining on Coat	0	2	7	0	1	4
Respiration Problems	0	2	6	0	0	4
Excess Salivation	0	1	6	0	1	3
Wet Coat on Body	0	2	6	0	0	2
Piloerection	0	1	5	0	0	2
Subdued Behaviour	0	0	4	0	1	3
Unkempt Coat	0	1	4	0	0	0
Pale/Discoloured Skin	0	0	3	0	0	1
Pale Eye(s)	0	0	2	0	0	1
Dark Eye(s)	0	1	2	0	0	1
Cold Skin on Body	0	0	2	0	0	0
Dark Skin on Body	0	0	2	0	0	0
Prostrate	0	0	1	0	0	3
Muscle Twitching/Spasm	0	0	1	0	0	0
Swollen Abdomen	0	0	1	0	0	0
Body Held Low	0	0	1	0	0	0
Mydriasis in Eye(s)	0	0	0	0	0	2
Resuscitation	0	0	0	0	0	1
Hunched Posture	0	1	0	0	0	0
Convulsions	0	0	0	0	1	0

Note: These numbers represent the number of animals in all groups showing the sign at any time during the dosing period.
Group 3 animals, the dose level was reduced from 120 mg Lu 26-054.$kg^{-1}.day^{-1}$ to 100 Lu 26-054.$mg.kg^{-1}.day^{-1}$ on Day 27.
Group 6 animals, the dose level was reduced from 160 mg Lu 10-171.$kg^{-1}.day^{-1}$ to 100 Lu 10-171.$mg.kg^{-1}.day^{-1}$ on Day 9

Figure 26, from page 41 of Report 99358

Females

Observation/Finding	Group/Dose Level (mg Lu 26-054/ Lu 10-171.kg^{-1}.day^{-1})					
	1 (0)	2 (80)	3 (120/100)	4 (80)	5 (120)	6 (160/100)
Number of Animals	0	0	4	0	3	3
Excess Salivation	0	0	4	0	3	3
Hunched Posture	0	0	4	0	3	3
Wet Coat on Body	0	0	4	0	3	2
Staining on Coat	0	0	4	0	3	3
Unkempt Coat	0	0	4	0	3	1
Respiration Problems	0	0	3	0	3	3
Piloerection	0	0	3	0	3	3
Swollen Abdomen	0	0	2	0	0	0
Rolling Gait	0	0	1	0	3	3
Pale/Discoloured Skin	0	0	1	0	2	2
Eye(s) Encrusted	0	0	1	0	2	1
Dark Eye(s)	0	0	1	0	1	0
Muscle Twitching/Spasm	0	0	0	0	3	2
Resuscitation	0	0	0	0	0	2
Subdued Behaviour	0	0	0	0	2	3
Tremors in Cage	0	0	0	0	1	2
Abnormal Vocalisation in Hand/Cage	0	0	0	0	0	2
Staggering	0	0	0	0	2	2
Prostrate	0	0	0	0	1	3
Pale Eye(s)	0	0	0	0	0	1
Lacrimating Eye(s)	0	0	0	0	0	1
Mydriasis in Eye(s)	0	0	0	0	0	1
Reduced Activity	0	0	0	0	0	1

Note: These numbers represent the number of animals in all groups showing the sign at any time during the dosing period.
Group 3 animals, the dose level was reduced from 120 mg Lu 26-054.kg^{-1}.day^{-1} to 100 Lu 26-054.mg.kg^{-1}.day^{-1} on Day 27.
Group 6 animals, the dose level was reduced from 160 mg Lu 10-171.kg^{-1}.day^{-1} to 100 Lu 10-171.mg.kg^{-1}.day^{-1} on Day 9

Figure 27, from page 42 of Report 99358

Clinical signs in non-decedents

Males

Observation/Finding	Group/Dose Level (mg Lu 26-054/ Lu 10-171.kg^{-1}.day^{-1})					
	1 (0)	2 (80)	3 (120/100)	4 (80)	5 (120)	6 (160/100)
Number of Animals	40	8	13	10	19	15
No abnormalities detected	40	0	0	0	0	0
Staining on Coat	0	6	13	10	19	15
Excess Salivation	0	7	11	10	19	14
Respiration Problems	0	0	6	0	12	15
Rolling Gait	0	0	0	0	6	13
Wet Coat on Body	0	6	10	6	17	11
Unkempt Coat	0	0	4	2	17	11
Hunched Posture	0	0	0	1	12	11
Subdued Behaviour	0	0	1	0	11	14
Eye(s) Encrusted	0	0	2	1	10	6
Eye(s) Lacrimating	0	0	0	0	10	4
Piloerection	0	0	1	0	4	6
Discoloured/Pale Skin	0	0	0	0	2	2
Coat Greasy	0	0	0	0	2	1
Tremors in Hand	0	0	0	0	1	0
Scabbing on Body	0	0	0	0	1	0
Dark Skin	0	0	0	0	1	0
Body Held Low	0	0	0	0	0	3
Prostrate	0	0	0	0	0	2
Convulsions	0	0	0	0	0	1
Pale Eye(s)	0	0	0	0	0	1
Resuscitation	0	0	0	0	0	1
Tremors in Cage	0	0	0	0	0	1
Dark Eye(s)	0	0	1	0	0	0
Eye(s) Bulging	0	0	0	1	0	0
Mydriasis in Eye(s)	0	0	0	1	0	0
Cold Skin	0	0	1	0	0	0

Note: These numbers represent the number of animals in all groups showing the sign at any time during the dosing period.
Group 3 animals, the dose level was reduced from 120 mg Lu 26-054.kg^{-1}.day^{-1} to 100 Lu 26-054.mg.kg^{-1}.day^{-1} on Day 27.
Group 6 animals, the dose level was reduced from 160 mg Lu 10-171.kg^{-1}.day^{-1} to 100 Lu 10-171.mg.kg^{-1}.day^{-1} on Day 9

Figure 28, from page 39 of Report 99358

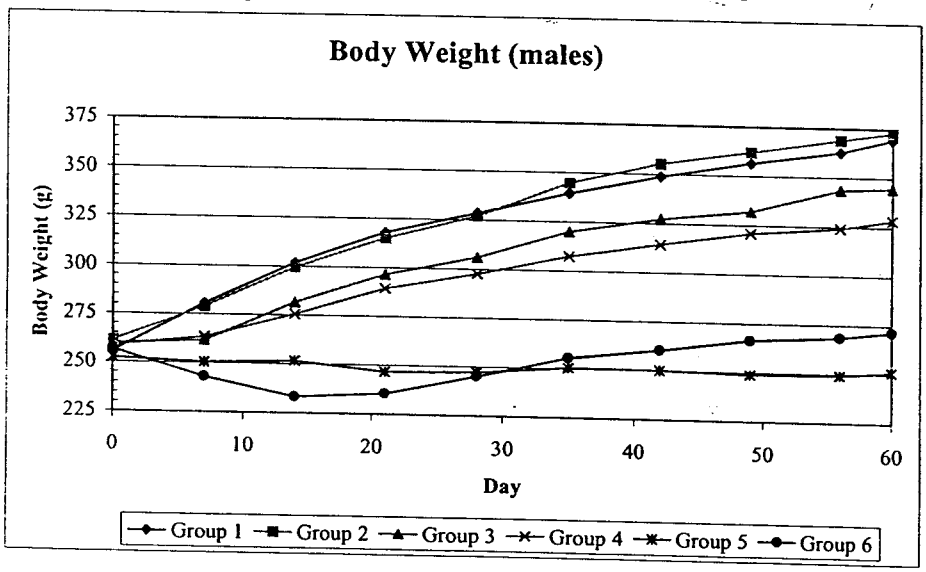
Females

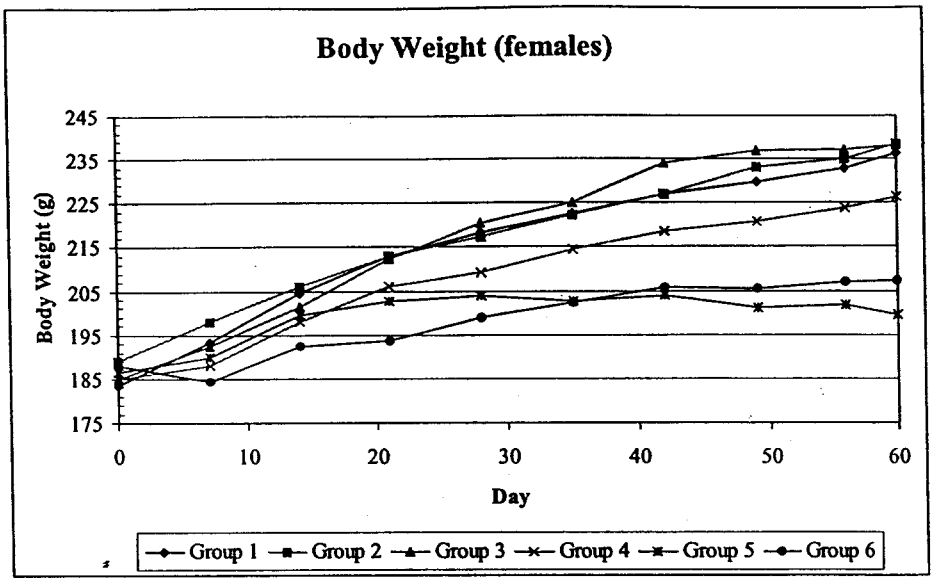
Observation/Finding	Group/Dose Level (mg Lu 26-054/ Lu 10-171.kg^{-1}.day^{-1})					
	1 (0)	2 (80)	3 (120/100)	4 (80)	5 (120)	6 (160/100)
Number of Animals	40	10	16	10	17	17
No abnormalities detected	40	0	0	0	0	0
Excess Salivation	0	10	16	9	17	17
Staining on Coat	0	9	14	10	17	17
Hunched Posture	0	4	7	4	16	15
Wet Coat on Body	0	5	12	7	15	14
Respiration Problems	0	0	8	0	15	9
Unkempt Coat	0	5	9	5	13	9
Eye(s) Encrusted	0	0	2	1	13	5
Subdued Behaviour	0	0	0	0	11	15
Rolling Gait	0	1	2	0	11	15
Eye(s) Lacrimating	0	0	1	1	9	2
Piloerection	0	1	5	0	7	9
Abdomen Swollen	0	0	4	1	7	6
Mydriasis in Eye(s)	0	0	0	0	3	5
Body Held Low	0	0	0	0	3	2
Discoloured/Pale Skin	0	2	2	0	2	2
Muscle Twitching/Spasm	0	0	0	0	2	1
Dark Eye(s)	0	1	0	0	1	3
Prostrate	0	0	0	0	1	2
Pale Eye(s)	0	0	0	0	1	2
Abnormal Vocalisation In hand	0	1	1	0	1	2
Tremors in Cage	0	0	0	0	1	0
Staggering	0	0	0	0	1	0
Coat Greasy	0	0	0	0	0	2
Resuscitation	0	0	0	0	1	2
Convulsions	0	0	0	0	0	1
Walking on Tip Toes	0	0	0	0	0	1

Note: These numbers represent the number of animals in all groups showing the sign at any time during the dosing period.
Group 3 animals, the dose level was reduced from 120 mg Lu 26-054.kg^{-1}.day^{-1} to 100 Lu 26-054.mg.kg^{-1}.day^{-1} on Day 27.
Group 6 animals, the dose level was reduced from 160 mg Lu 10-171.kg^{-1}.day^{-1} to 100 Lu 10-171.mg.kg^{-1}.day^{-1} on Day 9

Figure 29, from page 40 of Report 99358

- Body weights





- Food consumption Not done
- Ophthalmoscopy Not done
- Electrocardiography Not done
- Hematology Not done
- Clinical chemistry Not done
- Urinalysis Not done
- Organ Weights

	Sex	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Absolute Weight	Male	1.19	1.27 (107%)	**1.89 (159%)**	**0.98 (82%)**	**1.03 (87%)**	**0.97 (82%)**
	Female	0.88	**0.99 (113%)**	**1.47 (167%)**	0.81 (92%)	0.86 (98%)	0.82 (93%)
Relative Weight	Male	1.12	1.19 (107%)	**1.86 (166%)**	0.98 (88%)	1.16 (104%)	1.07 (96%)
	Female	0.83	0.95 (114%)	**1.42 (171%)**	0.81 (98%)	0.97 (117%)	0.89 (107%)

- Gross pathology Figures in **Bold** significantly different ($p<0.05$) from control by Fisher Exact test; only survivors and combined groups evaluated statistically.

Males		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Heart, Enlarged	Survivors	0/40	0/8	**6/13**	0/10	0/19	0/15
	Decedents	---	2/2	2/7	---	0/1	0/5
	Combined	0/40	**2/10**	**8/20**	0/10	0/20	0/20
Heart, Pale	Survivors	0/40	0/8	2/13	0/10	0/19	0/15
	Decedents	---	1/2	3/7	---	1/1	1/5
	Combined	0/40	1/10	**5/20**	0/10	1/20	1/20
Heart, Mass	Survivors	0/40	0/8	0/13	0/10	0/19	0/15
	Decedents	---	0/2	1/7	---	0/1	0/5
	Combined	0/40	0/10	1/20	0/10	0/20	0/20
Heart, Flaccid	Survivors	0/40	0/8	0/13	0/10	0/19	0/15
	Decedents	---	0/2	1/7	---	0/1	0/5
	Combined	0/40	0/10	1/20	0/10	0/20	0/20
Heart, Thin Atrial Wall	Survivors	0/40	0/8	0/13	0/10	0/19	0/15
	Decedents	---	0/2	1/7	---	0/1	0/5
	Combined	0/40	0/10	1/20	0/10	0/20	0/20
Liver, Dark and/or Enlarged	Survivors	0/40	0/8	0/13	0/10	0/19	0/15
	Decedents	---	1/2	3/7	---	0/1	0/5
	Combined	0/40	1/10	**3/20**	0/10	0/20	0/20
Lung, Darkened	Survivors	0/40	0/8	0/13	0/10	0/19	0/15
	Decedents	---	1/2	2/7	---	0/1	0/5
	Combined	0/40	1/10	2/20	0/10	0/20	0/20
Thoracic/Abdominal Cavity, Contains Fluid	Survivors	0/40	0/8	1/13	0/10	0/19	0/15
	Decedents	---	0/2	5/7	---	0/1	0/5
	Combined	0/40	0/10	**6/20**	0/10	0/20	0/20
Lymph Node, Enlarged and/or darkened	Survivors	0/40	0/8	1/13	0/10	0/19	0/15
	Decedents	---	0/2	4/7	---	0/1	0/5
	Combined	0/40	0/10	**5/20**	0/10	0/20	0/20
Females		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Heart, Enlarged	Survivors	0/40	0/10	**6/16**	0/10	0/17	0/17
	Decedents	---	---	3/4	---	0/3	0/3
	Combined	0/40	0/10	**9/20**	0/10	0/20	0/20
Heart, Pale	Survivors	2/40	0/10	1/16	0/10	1/17	2/17
	Decedents	---	---	1/4	---	0/3	0/3
	Combined	2/40	0/10	2/20	0/10	1/20	2/20
Thoracic/Abdominal Cavity, Contains Fluid	Survivors	0/40	0/10	0/16	0/10	0/17	0/17
	Decedents	---	---	3/4	---	0/3	0/3
	Combined	0/40	0/10	**3/20**	0/10	0/20	0/20
Lymph Node, Enlarged and/or darkened	Survivors	0/40	0/10	**3/16**	0/10	0/17	1/17
	Decedents	---	---	3/4	---	0/3	0/3
	Combined	0/40	0/10	**6/20**	0/10	0/20	1/20

- Histopathology Figures in **Bold** significantly different ($p<0.05$) from control by Fisher Exact test; only survivors and combined groups evaluated statistically.

Males		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
No Heart Abnormalities	Survivors	37/40	7/8	**6/13**	9/10	18/19	14/15
	Decedents	---	0/2	0/7	---	1/1	3/5
	Combined	37/40	7/10	**6/20**	0/10	19/20	17/20
Myocardial Hypertrophy	Survivors	0/40	1/8	**6/13**	0/10	0/19	0/15
	Decedents	---	1/2	2/7	---	0/1	0/5
	Combined	0/40	**2/10**	**8/20**	0/10	0/20	0/20
Chamber dilation	Survivors	0/40	0/8	7/13	0/10	0/19	0/15
	Decedents	---	2/2	5/7	---	0/1	0/5
	Combined	0/40	**2/10**	**12/20**	0/10	0/20	0/20
Thrombus	Survivors	0/40	0/8	2/13	0/10	0/19	0/15
	Decedents	---	2/2	3/7	---	0/1	0/5
	Combined	0/40	**2/10**	**5/20**	0/10	0/20	0/20
Ossification, chordae tendinae	Survivors	0/40	0/8	**5/13**	0/10	0/19	0/15
	Decedents	---	2/2	3/7	---	0/1	0/5
	Combined	0/40	**2/10**	**8/20**	0/10	0/20	0/20
Myocarditis, (includes epicarditis, endocarditis, and aortic valve inflammation)	Survivors	2/40	0/8	3/13	0/10	0/19	0/15
	Decedents	---	2/2	7/7	---	0/1	2/5
	Combined	2/40	2/10	**9/20**	0/10	0/20	2/20

Females		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
No Heart Abnormalities	Survivors	36/40	9/10	**6/16**	9/10	15/17	16/17
	Decedents	---	---	0/4	---	2/3	1/3
	Combined	36/40	9/10	**6/20**	9/10	17/20	17/20
Myocardial Hypertrophy	Survivors	0/40	0/10	**7/16**	0/10	0/17	0/17
	Decedents	---	---	2/4	---	0/3	0/3
	Combined	0/40	0/10	**9/20**	0/10	0/20	0/20
Chamber dilation	Survivors	0/40	0/10	**6/16**	0/10	0/17	0/17
	Decedents	---	---	4/4	---	0/3	0/3
	Combined	0/40	0/10	**10/20**	0/10	0/20	0/20
Thrombus	Survivors	0/40	0/10	0/16	0/10	0/17	0/17
	Decedents	---	---	1/4	---	0/3	0/3
	Combined	0/40	0/10	1/20	0/10	0/20	0/20
Ossification, chordae tendinae	Survivors	0/40	0/10	**6/16**	0/10	0/17	0/17
	Decedents	---	---	1/4	---	0/3	0/3
	Combined	0/40	0/10	**7/20**	0/10	0/20	0/20
Myocarditis, (includes epicarditis, endocarditis, and aortic valve inflammation)	Survivors	0/40	0/10	**7/16**	0/10	0/17	0/17
	Decedents	---	---	4/4	---	0/3	2/3
	Combined	0/40	0/10	**11/20**	0/10	0/20	2/20

- Toxicokinetics

S-Citalopram Toxicokinetics in Males

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	17,447	28,627	20,220	28,045	38,197
	7	14,612	34,973	18,582	26,378	27,528
	60	18,845	26,238	24,002	25.494	21,757
Cmax nmol/l	1	2,884	3,080	1,742	1,855	2,638
	7	1,901	2,549	1,604	1,921	1,796
	60	2,735	2,652	2,021	1,955	1,917
Tmax	1	0.5	1.5	1.5	1.5	0.5
	7	0.5	1.0	1.5	1.5	0.5
	60	1.0	1.0	1.0	1.5	1.5
Cmin nmol/l	1	134	461	220	637	904
	7	25	369	166	558	745
	60	54	104	291	509	566
Half Life	1	7.84	13.25	7.23	14.39	15.37
	7	3.25	6.91	6.05	12.12	19.03
	60	3.92	4.12	7.34	12.22	23.20

S-Citalopram Toxicokinetics in Females

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	18,878	27,797	14,774	19,983	33,761
	7	20,301	39,831	19,499	25,938	28,800
	60	26,282	28,402	30,549	29,431	20,893
Cmax nmol/l	1	2,914	3,476	1,548	2,666	2,795
	7	2,338	4,104	1,726	1,988	1,882
	60	3,306	3,018	2,515	1,840	1,645
Tmax	1	1.5	1.0	0.5	0.5	0.5
	7	1.5	0.5	0.5	1.5	1.5
	60	1.5	0.5	1.0	1.5	1.0
Cmin nmol/l	1	225	321	202	334	724
	7	98	732	247	723	1,018
	60	122	133	430	679	518
Half Life	1	10.63	8.38	8.59	11.65	13.80
	7	4.47	13.02	8.82	42.57	80.50
	60	4.64	4.34	9.59	15.46	17.92

R-Citalopram Toxicokinetics in Males

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	---	---	27,586	41,359	56,024
	7	---	---	54,825	101,808	114,541
	60	---	---	87,081	115,720	90,402
Cmax nmol/l	1	---	---	2,072	2,464	2,943
	7	---	---	3,573	5,317	5,692
	60	---	---	5,022	5,911	5,270
Tmax	1	---	---	1.5	6.0	0.5
	7	---	---	1.5	1.5	1.0
	60	---	---	1.0	1.5	1.5
Cmin nmol/l	1	---	---	386	1,197	1,732
	7	---	---	840	2,739	3,602
	60	---	---	1,829	3,587	2,984
Half Life	1	---	---	8.57	27.27	26.49
	7	---	---	8.34	18.33	26.84
	60	---	---	11.81	28.02	43.32

R-Citalopram Toxicokinetics in females

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	---	---	28,628	43,990	69,522
	7	---	---	66,303	96,733	124,166
	60	---	---	118,195	132,673	88,205
Cmax nmol/l	1	---	---	2,166	4,118	4,114
	7	---	---	4,384	5,090	6,635
	60	---	---	6,684	6,434	5,364
Tmax	1	---	---	1.5	0.5	3.0
	7	---	---	3.0	0.5	0.5
	60	---	---	1.0	1.5	1.0
Cmin nmol/l	1	---	---	421	834	1,907
	7	---	---	1,579	3,894	4,560
	60	---	---	3,129	4,846	2,633
Half Life	1	---	---	8.73	14.28	17.78
	7	---	---	16.84	ND	61.11
	60	---	---	19.11	60.88	22.2

Combined R- and S-Citalopram Toxicokinetics in Males

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	17,447	28,627	47,806	69,404	94,221
	7	14,612	34,973	73,408	128,187	142,069
	60	18,845	26,238	111,083	141,214	112,159
Cmax nmol/l	1	2,884	3,080	3,814	4,188	5,582
	7	1,901	2,549	5,177	7,238	7,370
	60	2,735	2,652	7,034	7,866	7,187
Tmax	1	0.5	1.5	1.5	1.5	0.5
	7	0.5	1.0	1.5	1.5	1.0
	60	1.0	1.0	1.0	1.5	1.5
Cmin nmol/l	1	134	461	606	1,834	2,636
	7	25	369	1,005	3,297	4,348
	60	54	104	2,120	4,097	3,550
Half Life	1	7.84	13.25	8.05	17.61	20.78
	7	3.25	6.91	7.76	17.26	34.93
	60	3.92	4.12	10.70	23.40	37.57

Combined R- and S-Citalopram Toxicokinetics in Females

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	18,878	27,797	43,402	63,973	103,282
	7	20,301	39,831	85,801	122,671	152,967
	60	26,282	28,402	148,459	162,088	109,098
Cmax nmol/l	1	2,914	3,476	3,704	6,783	6,674
	7	2,338	4,104	6,033	6,972	8,403
	60	3,306	3,018	9,066	8,258	7,009
Tmax	1	1.5	1.0	0.5	0.5	0.5
	7	1.5	0.5	3.0	1.5	0.5
	60	1.5	0.5	1.0	1.5	1.0
Cmin nmol/l	1	225	321	623	1,167	2,631
	7	98	732	1,826	4,617	5,579
	60	122	133	3,559	5,525	3,151
Half Life	1	10.63	8.38	8.68	13.36	16.30
	7	4.47	13.02	14.58	ND	63.86
	60	4.64	4.34	16.50	41.55	22.20

S-Demethylcitalopram (S-DCT) Toxicokinetics in Males

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	26,965	43,490	15,419	19,498	28,104
	7	44,420	87,517	31,380	56,604	58,454
	60	61,850	81,154	47,955	67,446	53,668
Cmax nmol/l	1	2,384	2,857	989	1,194	1,568
	7	2,932	4,532	1,577	2,522	2,759
	60	4,025	4,460	2,303	3,523	2,808
Cmin nmol/l	1	173	897	326	660	998
	7	266	2,336	867	2,188	2,094
	60	611	1,604	1,439	2,460	2,019

S-Demethylcitalopram (S-DCT) Toxicokinetics in Females

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	11,694	17,655	9,196	12,483	20,342
	7	27,775	63,837	25,274	37,148	43,244
	60	38,979	49,721	48,296	64,681	40,732
Cmax nmol/l	1	1,168	1,193	556	738	1,089
	7	1,627	3,182	1,384	1,980	2,092
	60	2,207	2,744	2,359	2,937	2,244
Cmin nmol/l	1	114	302	187	360	742
	7	464	2,016	832	1,579	1,565
	60	528	866	1,532	2,374	1,352

R-Demethylcitalopram (R-DCT) Toxicokinetics in Males

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	---	---	32,657	41,838	55,748
	7	---	---	68,838	108,260	113,662
	60	---	---	104,323	139,977	120,581
Cmax nmol/l	1	---	---	1,881	1,962	2,882
	7	---	---	3,187	5,104	5,257
	60	---	---	4,918	6,852	5,968
Cmin nmol/l	1	---	---	810	1,114	1,914
	7	---	---	2,206	3,350	4,422
	60	---	---	3,531	5,309	4,510

R-Demethylcitalopram (R-DCT) Toxicokinetics in Females

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	---	---	23,175	28,776	45,015
	7	---	---	63,502	82,065	87,420
	60	---	---	116,463	148,427	97,018
Cmax nmol/l	1	---	---	1,271	1,524	2,222
	7	---	---	3,113	4,371	4,359
	60	---	---	5,399	6,749	5,333
Cmin nmol/l	1	---	---	452	556	774
	7	---	---	2,492	2,551	3,196
	60	---	---	4,338	5,920	3,326

S-Didemethylcitalopram (S-DDCT) Toxicokinetics in Males

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	7,993	10,344	3,223	3,452	3,947
	7	13,707	23,366	6,821	8,142	9,013
	60	18,657	21,774	9,684	14,441	11,124
Cmax nmol/l	1	586	551	169	159	208
	7	747	1,249	344	410	439
	60	1,024	1138	448	744	573
Cmin nmol/l	1	140	196	75	59	75
	7	344	890	254	261	353
	60	512	781	353	545	387

S-Didemethylcitalopram (S-DDCT) Toxicokinetics in Females

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	2,664	3,067	1,473	1,767	1,811
	7	6,421	11,663	3,491	3,779	4,328
	60	8,727	12,506	7,947	11,790	6,969
Cmax nmol/l	1	150	154	94	87	100
	7	312	582	168	213	218
	60	407	628	383	552	398
Cmin nmol/l	1	37	27	15	17	0
	7	185	427	117	94	165
	60	318	434	299	422	240

R-Didemethylcitalopram (R-DDCT) Toxicokinetics in Males

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	---	---	18,577	20,202	23,017
	7	---	---	42,124	44,700	42,500
	60	---	---	53,187	74,564	57,996
Cmax nmol/l	1	---	---	872	1,306	1,303
	7	---	---	1,817	1,956	2,175
	60	---	---	2,390	3,423	2,963
Cmin nmol/l	1	---	---	215	195	222
	7	---	---	1,654	1,601	1,660
	60	---	---	2,027	2,602	2,141

R-Didemethylcitalopram (R-DDCT) Toxicokinetics in Females

	Day	80 mg/kg S-CT	120 mg/kg S-CT	80 mg/kg RS-CT	120 mg/kg RS-CT	160 mg/kg RS-CT
AUC nmol.h/l	1	---	---	10,120	11,450	14,812
	7	---	---	26,714	27,675	33,129
	60	---	---	49,885	77,569	44,933
Cmax nmol/l	1	---	---	515	655	978
	7	---	---	1,380	1,552	1,490
	60	---	---	2,501	3,549	2,451
Cmin nmol/l	1	---	---	62	68	61
	7	---	---	987	786	1,274
	60	---	---	1,953	2,990	1,545

Key Study Findings:

1. Cardiotoxicity was observed starting at 80 mg/kg in rats treated with S-citalopram. The lesion was characterized by myocarditis (generally mild) in all premature decedents and in 3/13 rats that survived at 120/100 mg/kg. In addition, chamber dilation was observed in 7/13 surviving rats and 5/7 premature decedents at 120/100 mg/kg. A low incidence of myocarditis was observed in rats treated with 160/100 mg/kg RS-citalopram. Similar findings were noted in female rats.
2. It appears that the toxicity (eg decreased weight gain, CNS clinical signs (rolling gait, subdued behavior)) observed in the RS-citalopram groups is related to factors other than cardiotoxicity, even at doses where equal doses of S-citalopram were administered (160 mg/kg RS-citalopram). Substantial decreases in body weight gain were observed at 120 and 160 mg/kg RS-citalopram. This effect was not observed at 120 mg/kg escitalopram.
3. The toxicokinetics suggest that the cardiotoxicity is a function of the S-enentiomer, since higher total citalopram levels were observed in the RS-citalopram groups with only a low incidence of cardiotoxicity.

Overall Toxicology Summary:

The main target organ was the heart. S-citalopram at doses between 80 and 120 mg/kg caused cardiac failure, with males being more affected than females. There was also evidence of phospholipidosis (vacuolated lymphocytes and epididymides) at doses as low as 40 mg/kg. Clinical signs (salivation) were observed as doses as low as 20 mg/kg. The NOEL was 10 mg/kg/day.

Addendum list:

Addendum 1

Histopathology Inventory for IND #

Study	90 Day	28 Day	60 Day		
Species	Rat	Rat	Rat		
Adrenals	✔*	✔*			
Aorta	✔*	✔*			
Bone Marrow smear	✔	✔#			
Bone (femur)					
Brain	✔*	✔*			
Cecum	✔	✔			
Cervix					
Colon	✔	✔			
Duodenum	✔	✔			
Epididymis	✔*	✔*			
Esophagus	✔	✔			
Eye	✔	✔			
Fallopian tube					
Gall bladder					
Gross lesions					
Harderian gland					
Heart	✔*	✔*	✔*		
Hyphophysis					
Ileum	✔	✔			
Injection site					
Jejunum	✔	✔			
Kidneys	✔*	✔*			
Lachrymal gland					
Larynx					
Liver	✔*	✔*			
Lungs	✔*	✔*			
Lymph nodes, cervical					
Lymph nodes mandibular					
Lymph nodes, mesenteric	✔	✔			
Mammary Gland	✔	✔			
Nasal cavity	✔#	✔=			
Optic nerves	✔	✔			
Ovaries	✔*	✔*			
Pancreas	✔	✔			
Parathyroid	✔*	✔*			
Peripheral nerve					
Pharynx					
Pituitary	✔*	✔*			
Prostate	✔*	✔*			
Rectum	✔	✔			
Salivary gland	✔*	✔*			
Sciatic nerve	✔	✔			
Seminal vesicles	✔	✔			
Skeletal muscle	✔	✔			
Skin	✔	✔			
Spinal cord	✔	✔			
Spleen	✔*	✔*			
Sternum	✔	✔			
Stomach	✔	✔			
Testes	✔*	✔*			
Thymus	✔*	✔*			
Thyroid	✔*	✔*			
Tongue	✔	✔			
Trachea	✔	✔			
Urinary bladder	✔	✔			
Uterus	✔*	✔*			
Vagina	✔	✔			
Zymbal gland					

organ weight obtained # organ obtained but not examines.

REPRODUCTIVE TOXICOLOGY

Oral (Gavage) Developmental Toxicity Study of R/S Citalopram [Citalopram Hydrobromide (Lu 10-171-B)] and S Citalopram [S-Citalopram Oxalate (Lu 26-054-O)] in Rats.

Study No: and number: GLP 3691/855
Volume and Page Number: Volume 23, page 5-03889
Site and testing facility: [redacted]

GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: 002
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- Species/strain: Rat, Crl:CDBR VAF/Plus (Sprague-Dawley)
- Doses employed: 56, 112, 150 mg/kg, 70 mg/kg RS-citalopram
- Route of Administration: Oral (Gavage)
- Study Design: Dams dosed Gestation Days 6 through 17
- Number of animals/sex/dosing group: 25 /dose
- Parameters and endpoints evaluated: Maternal toxicity and fetal abnormalities
- Statistical evaluations: Bartletts Tests, Kruskal-Wallis Test, ANOVA, Dunn's, Fisher's, Dunnett's

Results:

- Clinical signs: Incidence and number of observations

Clinical Sign	Control	S-Citalopram			RS-Citalopram
	0 mg/kg	56 mg/kg	112 mg/kg	150 mg/kg	70 mg/kg
Excess Salivation	0/25 (0/375)	4/25 (94/375)	18/25 (41/370))	21/25 (69/375)	14/25 (21/375)
Urine Stained Abdominal Fur	0/25 (0/375)	0/25 (0/375)	9/25 (14/370)	14/25 (51/375)	2/25 (6/375)
Ungroomed Coat	0/25 (0/375)	4/25 (4/375)	7/25 (12/370)	7/25 (11/375)	7/25 (9/375)
Rales	0/25 (0/375)	0/25 (0/375)	3/25 (4/370)	5/25 (10/375)	0/25 (0/375)
Alopecia	0/25 (0/375)	1/25 (14/375)	2/25 (21/370)	5/25 (53/375)	5/25 (46/375)
Brown or Red Perioral Substance	0/25 (0/375)	1/25 (1/375)	0/25 (0/370)	4/25 (6/375)	1/25 (1/375)

- Mortality: one 112 mg/kg dam died due to intubation error; no other deaths

Body weight:

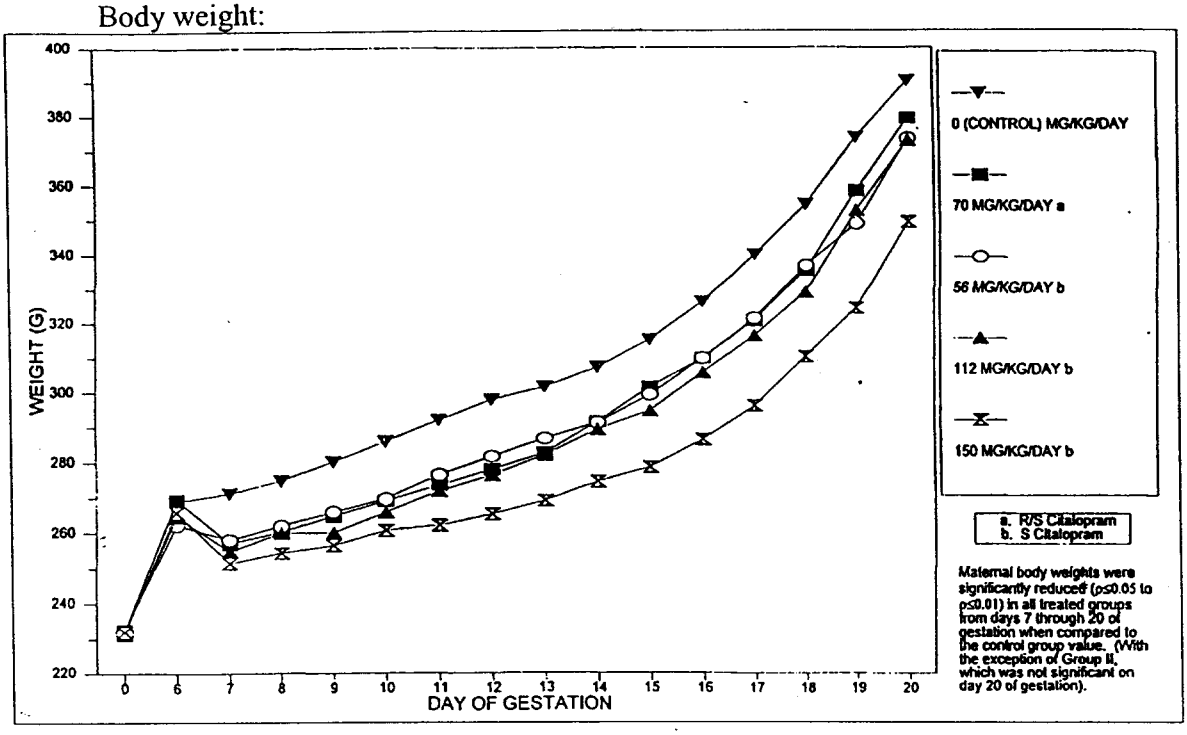


Figure 30, from page 43 of Report 912-016

- Food consumption: Decreased food consumption at all dose levels during dosing
  - Toxicokinetics: Not Done
  - Embryo-fetal Development
  - In-life observations:

Fetal body wt decreased 7 and 12% at 112 and 150 mg/kg; fetal weight decreased 9% in RS-citalopram

No effects on fetal viability or number of implantations

  - Terminal and Necroscopic evaluations:
    - Dams: all placentae appeared normal
  - Offspring:

Alterations

0 mg/kg- 1/335 had no anal opening

150 mg/kg- 1/336 had cleft palate

112 mg/kg- 1/333 had a short tail

70 mg/kg RS-Citalopram- 1/384 had multiple external abnormalities

Variations

Variation	Litter/ Fetus	0 mg/kg	56 mg/kg S-citalopram	112 mg/kg S-citalopram	150 mg/kg S-citalopram	70 mg/kg RS-citalopram
Sternal Centra Variations	Litter	1 (4.2%)	4 (16.7%)	3 (13.0%)	**8 (32.0%)**	3 (12.0%)
	Fetus	1 (0.6%)	4 (2.2%)	4 (2.3%)	**15 (8.7%)**	4 (2.0%)
Sternal Centra, Incomplete Ossification	Litter	0 (0%)	3 (12.5%)	2 (8.7%)	**6 (24.0%)**	2 (8.0%)
	Fetus	0 (0%)	3 (1.7%)	3 (1.7%)	**8 (4.6%)**	2 (1.0%)
Sternal Centra, Not ossified	Litter	1 (4.2%)	1 (4.2%)	1 (4.3%)	4 (16.0%)	2 (8.0%)
	Fetus	1 (0.6%)	1 (0.6%)	1 (0.6%)	**7 (4.1%)**	2 (1.0%)

Values in **Bold** significantly different from control at $p<0.05$

Number of Fetal Ossification Sites

Site	0 mg/kg	56 mg/kg S-citalopram	112 mg/kg S-citalopram	150 mg/kg S-citalopram	70 mg/kg RS-citalopram
Vertebrae, Caudal	4.87	4.88	4.51	**4.26**	**4.39**
Sternal Centers	3.72	3.67	**3.50**	**3.35**	**3.50**
Forelimb Metacarpals	3.68	3.74	3.51	**3.40**	**3.43**

Values in **Bold** significantly different from control at $p<0.05$

Summary and Evaluation:

1. Clinical signs and decreased body weight gain suggest mild maternal toxicity indicate that the selected dose was sufficiently high.
2. Fetal weight was decreased 7 and 12% at 112 and 150 mg/kg, respectively
3. No significant increase in abnormalities
4. Main adverse effects at 112 mg/kg was reduced number of sternal ossification sites
5. Main adverse effects at 150 mg/kg were delayed ossification the sternum and reduced number of ossification sites in the caudal vertebra, sternal centers and metacarpals
6. 70 mg/kg RS-Citalopram was not teratogenic in this study, but 112 mg/kg was teratogenic in two previous studies; 56 mg/kg was the NOEL for teratogenicity; why 70 mg/kg was selected as the dose in this study is unclear.

Oral (Gavage) Combined Dosage-Range and Full Developmental and Perinatal/Postnatal Reproduction Toxicity Study of R/S Citalopram [Citalopram Hydrobromide (Lu 10-171-B)] and S Citalopram [S-Citalopram Oxalate (Lu 26-054-O)] in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.: 912-018
Volume #, and page #: Volume 24 / page 5-04454
Conducting laboratory and location: [redacted]

Date of study initiation: February 2, 2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: [redacted]
Formulation/vehicle: Saline

Methods:

Species/strain: Rat, Crl:CD(SD)IGS BR VAF/Plus
Doses employed:

Dosage Group	Descriptor	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Female Rats	Assigned Numbers
I	Control	0 (Vehicle)[b]	0	10	25	3201-3225
II	R/S Citalopram	12	1.2	10	25	3226-3250
III	R/S Citalopram	48	4.8	10	25	3251-3275
IV	S Citalopram	6	0.6	10	25	3276-3300
V	S Citalopram	12	1.2	10	25	3301-3325
Vi	S Citalopram	24	2.4	10	25	3326-3350
VII	S Citalopram	48	4.8	10	25	3351-3375

a. Dosages were calculated as the free base using a correction factor of 1.25 for R/S Citalopram and a correction factor of 1.28 for S Citalopram.
b. This is referred to as 0 (Control) throughout the tables.

Figure 31, from page II-12, Report 912-018

Route of administration: Oral Gavage
Study design: Dams were dosed from Gestation Day 1 through post partum day 20; 25 F: males and females/group were evaluated for various parameters.
Number/sex/group: 25 females/group (F_0);
Parameters and endpoints evaluated:
PP Day 24- Passive Avoidance Testing, 2 sessions 1 week apart
PP Day 28, 39- Sexual maturation of females and males
PP Day 70- Watermaze testing
PP Day 90- Reproductive Capacity

Results:

Mortality: none

Clinical signs:

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
				Gestation			
Salivation	0/25 0/550	1/25 1/556	8/25 15/565	0/25 0/552	0/25 0/553	6/25 12/558	10/25 14/554
Alopecia	0/25 0/550	0/25 0/556	0/25 0/565	0/25 0/552	0/25 0/553	0/25 0/558	2/25 35/554
				Lactation			
Salivation	0/25 0/525	0/24 0/504	0/23 0/427	0/24 0/504	0/24 0/504	1/23 2/483	4/24 4/504
Alopecia	0/25 0/525	0/24 0/504	0/23 0/427	0/24 0/504	0/24 0/504	0/23 0/483	1/24 21/504

Body weight: Decreased body weight gain in 48 mg/kg RS-Citalopram and S-Citalopram groups (-13.3 and 9.7%, respectively). Decreased body weight in F1 males at 48 mg/kg RS-citalopram and S-citalopram. Decreased body weight in F1 females in 48 mg/kg RS-citalopram.

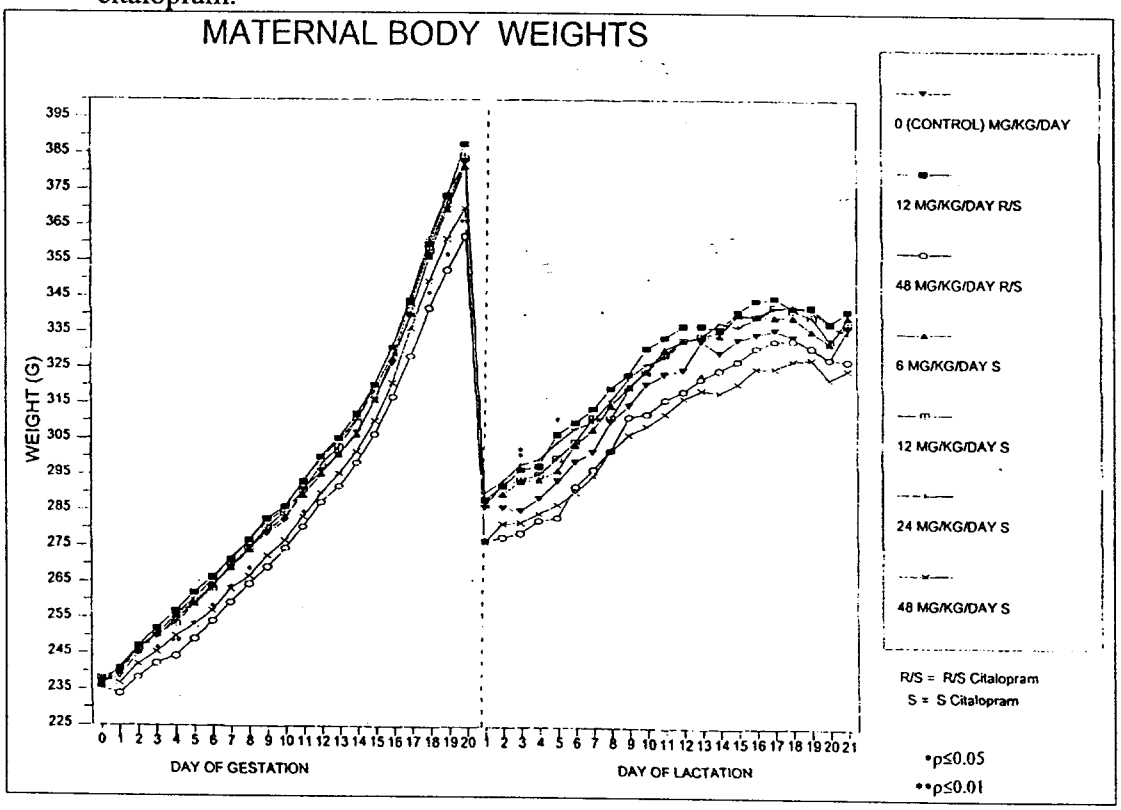


Figure 32, from page A-1 in Report 912-018

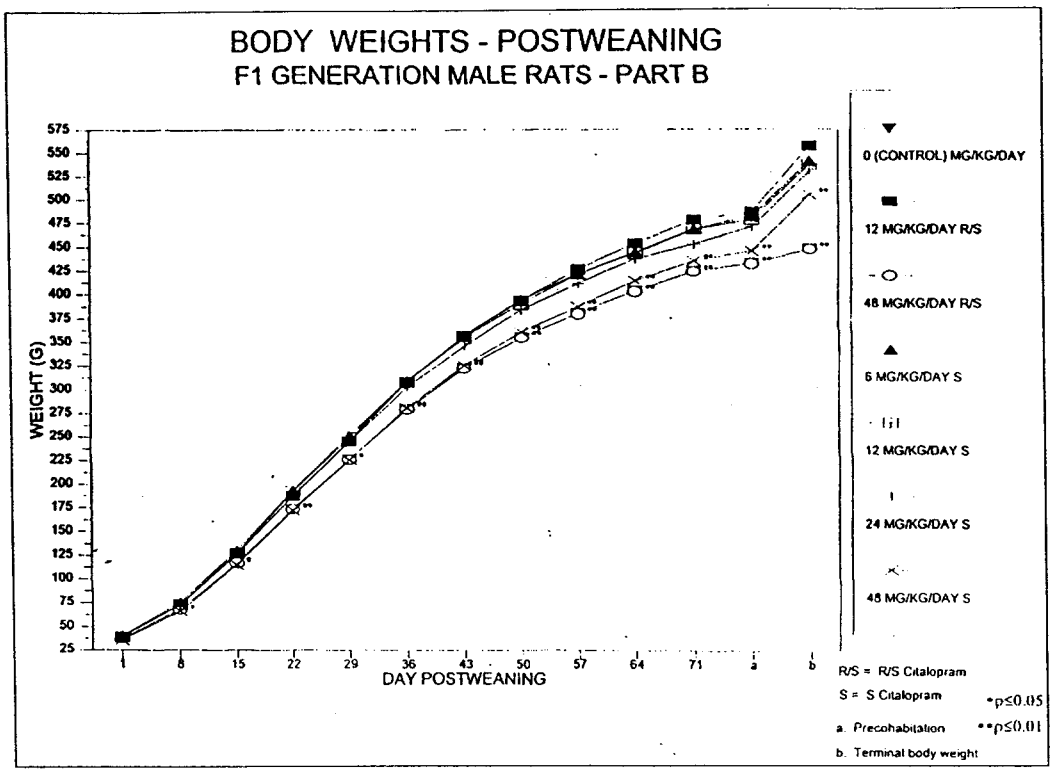


Figure 33, from page A-2 in Report 912-018

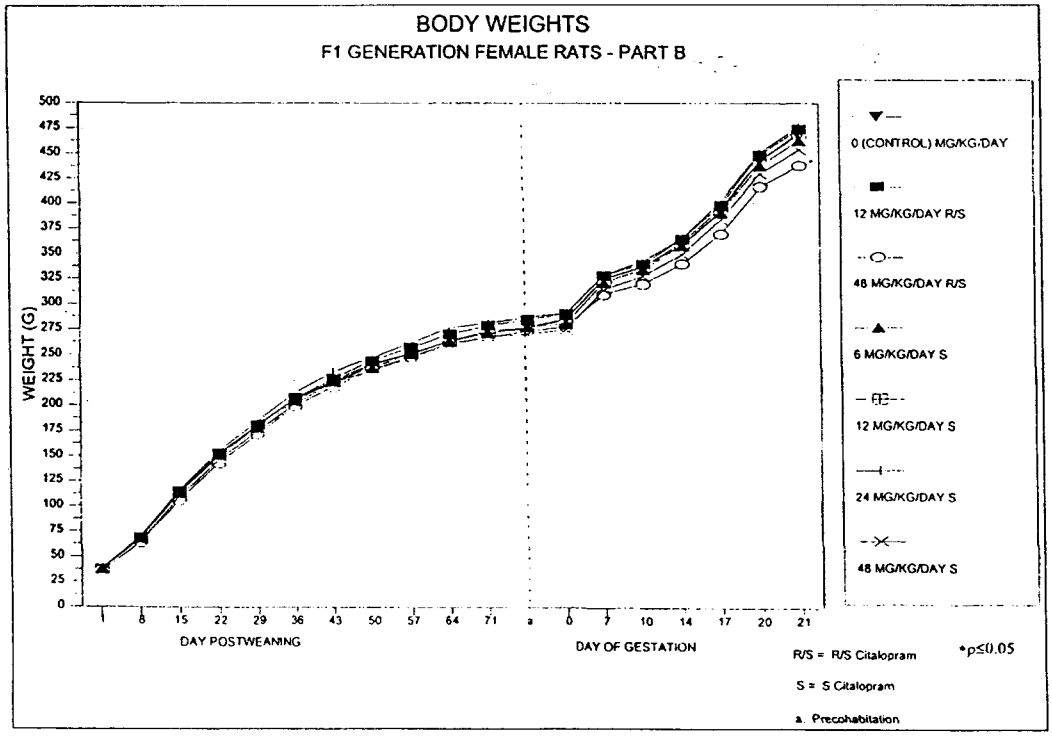
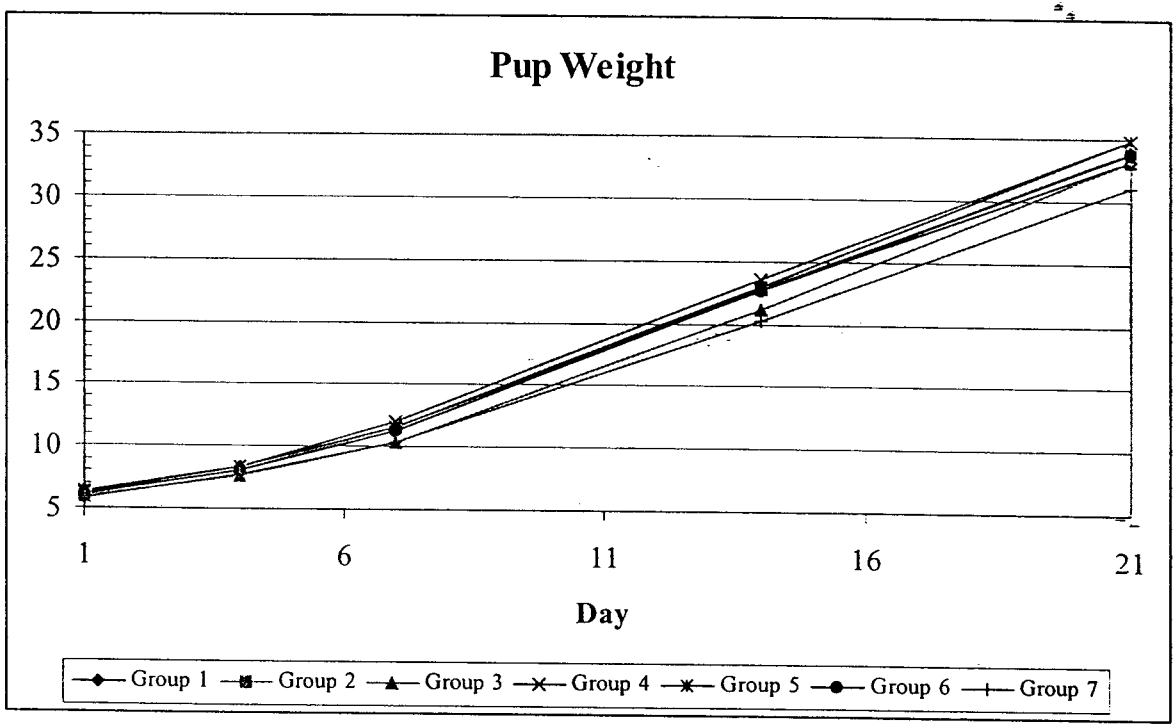


Figure 34, from page A-3 from Report 912-018

Food consumption:
Toxicokinetics:
In-life observations:
Dams:
Offspring:

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Dams with stillborn pups	2/25	1/24	**8/23**	3/24	0/24	1/23	3/24
Stillborn pups	2/371 (0.5%)	1/364 (0.0%)	**11/311 (3.5%)**	3/348 (0.9%)	0/351 (0.0%)	1/340 (0.3%)	4/367 (1.1%)
Dams with all pups dying PP Days 1-4	0/25	0/24	**3/23**	0/24	0/24	0/23	0/24
Viability Index	98.1	96.2	**74.8**	99.4	98.6	**93.5**	**94.2**
Lactation Index	98.6	98.8	97.3	99.4	97.4	97.8	**93.5**

Decreased pup weight at 48 mg/kg RS-citalopram and S-citalopram



No effects on day of sexual maturation in males or females. No adverse effects on passive avoidance learning. Increased number of F1 males in the 48 mg/kg RS citalopram group failed to learn the watermaze task. No adverse effects on reproductive performance.

Terminal and necroscopic evaluations:
Dams: No abnormalities
Offspring: No abnormalities

Key Results

1. 48 mg/kg RS-citalopram and S-citalopram caused comparable decreases in body weight of dams treated throughout gestation and lactation.
2. Decreased pup weight was observed at 48 mg/kg RS-citalopram and S-citalopram throughout lactation.
3. Decreased pup viability (as evidenced by decreased viability and lactation indices) were observed at 48 mg/kg RS-citalopram and 24 and 48 mg/kg S-citalopram.
4. Decreased body weight was observed in male pups throughout the post weaning period.
5. Decreased performance of males in the watermaze test in the 48 mg/kg RS-citalopram group, but no effect on S-citalopram males or females. No significant effects on passive avoidance learning at any dose level.
6. No effects on reproductive performance in the F1 generation.

Labeling Recommendations:
It is recommended that the labeling include results from the racemic mixture. Pregnancy category C is recommended to reflect the effects of citalopram on the pregnant animal and the lack of human studies.

OVERALL SUMMARY AND EVALUATION

Drug History

Forest Laboratories, Inc proposed a preclinical development program for escitalpram under their IND for racemic citalopram (——— submission 072, dated February 27, 1998) that was agreed to by the Division of Neuropharmacological Drug Products (FDA response letter dated April 22, 1998). The proposed program was based upon the FDA's Policy Statement for the Development of New Stereoisomeric Drugs (published May 1, 1992, revised January 3, 1997). The pivotal studies included comparative 4-and 13-week general toxicity studies and an embryo-fetal developmental toxicity study in the rat. Under the provisions of this policy, it was not necessary to conduct preclinical trials in a second non-rodent species nor was necessary to conduct carcinogenicity studies. The full reproductive toxicity battery was not required. An in vitro cytochrome P450 metabolism profiling study of escitalopram in human microsomes was conducted to assess the potential for drug-drug interactions. In the course of the preclinical development program, an unexpected finding of cardiac toxicity was observed in the 13 week study (——— Serial Submission 028, dated February 8, 2000). An additional bridging study using higher doses of racemic citalopram and incorporating additional toxicokinetic evaluations was requested (FDA Action Letter dated April 17, 2000). This study was received February 2001 (——— Serial Submission 117, dated February 9, 2001). In the course of the development of this drug, the sponsor has conducted all of the FDA requested studies. As part of the NDA submission, the sponsor also submitted studies requested by European drug authorities.

Safety Evaluation

The purpose of the studies submitted under this IND is to compare the toxicity of the S-enantomer to the racemic mixture. The racemic mixture is 50% S-citalopram and R-citalopram.

Pharmacology Studies

Racemic citalopram is a selective serotonin reuptake inhibitor which is approved as an antidepressant. Pharmacology have studies demonstrated that escitalopram is a more potent inhibitor of serotonin uptake than the R enantiomer of citalopram. The IC50 value for escitalopram was 1.5 nM versus 250 nM for the R enantiomer. Escitalopram was also more active than the R enantiomer in preclinical models of depression (forced swimming test in mice, vocalization following shock in rats and reducing aggressive behavior in mice). In addition, escitalopram was relatively specific inhibitor of serotonin uptake over norepinephrine (IC50 = 2,500 nM) or dopamine (IC50 = 65,000 nM). In a receptor screening assay, the binding of escitalopram was less than 50% at 1 uM except at the sigma-1 receptor (85%). These data suggest that escitalopram is a relatively selective serotonin reuptake inhibitor with potential to be a antidepressant in humans.

Cardiovascular Safety Pharmacology Studies

Due to concerns about the potential cardiotoxicity of escitalopram, a series of safety pharmacology studies have been performed. The studies included in vivo and in vitro assessments of effects on heart function.

In vitro studies using isolated perfused guinea pig heart suggest that escitalopram has the potential to affect cardiac function. In the isolated guinea pig heart preparation, a dose dependent negative inotropic response was observed between 500 nM (lowest concentration tested) and 2,500 nM escitalopram; a small increase in QT interval was also observed in this study (see study 99288, page 13). In another study using perfused guinea pig heart, a dose dependent negative inotropic effect was observed between 500 nM and 2,500 nM, but only the decrease at 2,500 nM was statistically significant (see study 99557, page 14). The slight increase in QT interval may be related to inhibition of rectifying potassium channel (Ikr) by escitalopram (see study 99558, page 15). In the cardiovascular study in conscious beagle dogs (study 99283, see page 11), the effect of escitalopram on cardiac contractility was not directly assessed, although decreased blood pressure was observed at 3 and 6 mg/kg infusions. This is consistent with a negative inotropic effects of the drug. No effects were observed on the QT interval in this study. These studies suggest that escitalopram has the potential to affect cardiac function.

Repeat Dose Bridging Studies of S-Citalopram and RS-Citalopram

One Year Toxicity Study on RS-Citalopram Alone

For purposes of comparison, it is useful to review the toxicity of RS-citalopram (abstracted from Robin Huff's review of NDA 20-822, see Appendix 1, page 79). In a one year dietary study, rats were administered 32, 60, and 120 mg/kg/day RS-citalopram. No increase in mortality was observed at 120 mg/kg/day. The primary adverse effects observed in the one year study were seizures and rigid tail. These effects appeared to occur later in the study. The rigid tails were first noted during the twelfth week of the study; the previous reviewer did not note when the seizures occurred, but they appeared to occur towards the end of the study. There was a dose dependent decrease in body weight gain at all doses (30, 18, and 6% less than control for 120, 60, and 32 mg/kg, respectively. Vacuolated lymphocytes were also observed at 60 and 120 mg/kg.

Four Week Rat Study

In the four week bridging study, rats were administered S-citalopram at doses of 5, 20, 40 or 60 mg/kg by gavage; two groups were also administered RS-citalopram at doses of 20 or 60 mg/kg. Decreased body weight gain was observed at 60 mg/kg with both enantiomers. The effects were more pronounced with the racemate than with the S-enantiomer. Likewise, clinical signs (salivation, mouth and muzzle staining, and unkempt coat) were observed at 20 mg/kg and above for both forms of the drug. There was no appreciable difference in the incidence of these clinical signs between these two formulations. Increased vacuolation of the epididymides was observed at 60 mg/kg for both forms of the drug; the severity of the effect was more severe in the RS-citalopram group. Likewise, an increase in the incidence of vacuolated lymphocytes was more severe in the RS-citalopram group.

In summary, the toxicity of the two enantiomers were similar and there was no particular evidence of any enantiomer specific toxicity in this study. The racemate appeared slightly more toxic, which may be due to a slightly longer half-life than the S-form.

13 Week Rat Study

In the 13 week study, rats were administered S-citalopram at doses of 10, 40 or 120 mg/kg by gavage; two groups were also administered RS-citalopram at doses of 5 or 60 mg/kg. Rats did not tolerate the 120 mg/kg dose regimen. Early mortality forced the reduction of the high dose to 80 mg/kg. Histology of early decedents revealed vacuolation of the epithelium of the epididymides and alveolar foamy macrophage accumulation in the lungs. Findings affecting the heart including atrial/ventricular thrombus, papillary muscle ossification or cartilaginous metaplasia, chamber dilation, progressive cardiomyopathy and myocarditis whilst findings in the liver and lungs included fibrosis, necrosis, congestion/hemorrhage. Atrophy and congestion were also noted in the pancreas and thymus. These pathological findings were considered to be indicative of cardiac failure. It was also noted that it was difficult to obtain blood samples from the tail vein of rats treated with 120 mg/kg S-citalopram. This failure was attributed to drug induced toxicity which caused decreased peripheral blood circulation.

In addition to the cardiac effects, there was also evidence of phospholipidosis (vacuolated lymphocytes and epididymides) at 40 mg/kg and above, however the incidence of vacuolated lymphocytes was not as great as that observed with 60 mg/kg of RS-citalopram. A dose dependent increase in the incidence of macrophage accumulation was observed in the lungs of rats treated with 40 mg/kg. A higher incidence of pulmonary macrophage accumulation was observed at 60 mg/kg RS-citalopram (14/20, combined sexes) than at 80 mg/kg of S-citalopram (9/28). Clinical signs (salivation) were observed as doses as low as 40 mg/kg in S-citalopram treated rats and at 60 mg/kg in RS-citalopram treated rats. Decreases in body weight gain were observed in male rats treated with 120 mg/kg S-citalopram (-18%)and 60 mg/kg RS-citalopram (-14%).

In summary, the toxicity of the 60 mg/kg RS-citalopram is similar to that observed in the one year toxicity study on RS-citalopram. This indicates that there is reasonable reproducibility of effects between the two studies. In this study, the 120 mg/kg S-citalopram dose induced an unacceptable level of deaths due to cardiac failure. The lower incidence of vacuolated lymphocytes implies that this effect may not be attributed entirely to the S enantiomer.

60 Day Rat Bridging Study

This study was conducted to evaluate blood levels of escitalopram associated with cardiotoxicity and to compare these blood levels to those observed in rats administered racemic citalopram. In this study, rats were administered 80 or 120 (lowered to 100 on Day 27 of the study) mg/kg escitalopram or 80, 120, 160 (lowered to 100 mg/kg on Day 9 of the study) of RS-citalopram. The 160 mg/kg RS-citalopram group provided 80 mg/kg S-citalopram, while the 80 and 120 mg/kg RS-citalopram provided equivalent citalopram doses to the escitalopram groups. Greater toxicity at nominally equivalent doses was observed in the escitalopram groups. Significantly higher mortality was observed in the 120 mg/kg escitalopram group then in the 120 mg/kg RS-citalopram group. Significant mortality was also observed at 160 mg/kg RS-citalopram. Decreased body weight was observed at 120 and 160 mg/kg RS-citalopram. Less severe body weight gain reductions were observed at 120 mg/kg escitalopram and 80 mg/kg RS-citalopram. A dose dependent increase in cardiotoxicity (primarily myocarditis) was observed at 80 and 120 mg/kg of S-citalopram, but not in RS-citalopram treated rats. The differences in toxicity appear to be due to differences in the toxicokinetics of the drugs. Higher Cmax values were observed with the escitalopram groups, while the RS-citalopram groups had higher AUC values characterized by lower Cmax values, but higher Cmin values.

Perinatal/Postnatal Reproductive Toxicity Bridging Study of S-Citalopram and RS-Citalopram in Rats

In this study, rats were exposed to 6, 12, 24 or 48 mg/kg S-citalopram from gestation day 1 through post partum day 20. Additional rats were exposed to 12 or 48 mg/kg RS-citalopram on the same days. Decreased body weights were observed in the dams exposed to 48 mg/kg S- or RS-citalopram. An increased number of stilborn pups was observed at 48 mg/kg RS-citalopram (11/311 pups compared to 2/371 in controls). A small increase non-statistically significant increase in stilborn pups was observed at 48 mg/kg S-citalopram (4/367). Decreased body weight was observed in F1 male, but not female, pups at 48 mg/kg S- or RS-citalopram. Increased pup mortality (as indicated by the number of pups surviving to post natal day 4 compared to number of pups born alive (viability index)) was noted at 24 and 48 mg/kg S-citalopram and at 48 mg/kg RS-citalopram. The decrease was more severe in the RS-citalopram treated dams (viability index=74.8%, compared to 98.1% in controls) than in S-citalopram treated dams (93.5 and 94.2 for 24 and 48 mg/kg S-citalopram, respectively). In addition, increased mortality was observed between post partum days 4 and 21 at 48 mg/kg S-citalopram (lactation index = 93.5% compared to 98.6% in controls). No effects were observed on reproductive performance, sexual development or learning at any dose level.

Although there is no exact duplicate of this study in the RS-citalopram submissions, the results are broadly similar with previous studies. In one study dams were treated with 16, 32, 64 or 128 mg/kg RS-citalopram from gestation day 17 through post natal day 7, the last day of the study. Maternal toxicity (decreased body weight gain and salivation) were observed at 64 and 128 mg/kg as was a decrease in pup viability (50% at 68 mg/kg and 0% at 128 mg/kg at post natal day 7). In another study, dams were treated with 4.8, 12.8, or 32 mg/kg RS-citalopram from gestation day 17 through past partum day 21. Increased pup mortality was observed at 32 mg/kg (survival index = 74.6% compared to 96.5% in controls). No effects were observed on reproductive performance of the F1 generation. In summary, both S-citalopram and RS-citalopram decrease the pup survival of treated dams.

Embryo-fetal Toxicity Bridging Study of S-Citalopram and RS-Citalopram in Rats

Segment 2 Reproductive Toxicity Study on RS-Citalopram Alone

Evidence of teratogenicity was found in both rat Segment II studies that used only RS-citalopram at doses of 40, 70, and 140 mg/kg. Mild maternal toxicity, including salivation, pupil dilation, and lethargy, were observed in most 140 mg/kg 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 140 mg/kg, mainly as a result of late resorptions. 140 mg/kg fetal weight was decreased to 87% of control. Abnormalities were increased at the high dose, 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 froin 11/22 140 mg/kg litters had abnormalities detected by free hand sectioning, and 29/177 in 12 of 22 140 mg/kg 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 140 mg/kg 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.

Segment 2 Reproductive Toxicity Bridging Study

In this study, dams were exposed to 56, 112, or 150 mg/kg S-citalopram or 70 mg/kg RS-citalopram. A dose-dependent increase in maternal toxicity was observed at all doses. Clinical signs included excess salivation (56 mg/kg and above), ungroomed coat (56 mg/kg and above), and urine stained abdominal fur (112 mg/kg and above). Transient decreases in body weight were also observed at all dose levels. Decreased fetal weight was observed at 112 (-7%) and 150 (-12%) mg/kg S-citalopram and 70 (-9%) mg/kg RS-citalopram. Associated with this fetotoxicity was a reduction in the number of ossification sites in the caudal vertebrae, sternal centers, and forelimb metacarpals at these doses. In addition, there was incomplete ossification of the sternal centra at 150 mg/kg S-citalopram only. No increase in the incidence of malformations was observed. In summary, 112 mg/kg S-citalopram was fetotoxic, however no increase in the incidence of malformations was observed.

Clinical Relevance of Safety Issues

The highest proposed human dose of escitalopram is 20 mg/day. At this dose, the estimated human Cmax is 42 ng/ml and the estimated AUC is 730 ng-hour/ml. Assuming that the patient weighs 60 kg, the equivalent dose for rats is 2 mg/kg/day.

The primary concern with S-citalopram is cardiotoxicity which has been observed at doses as low as 80 mg/kg/day in rats. No cardiotoxicity was observed at 40 mg/kg. The results of the bridging studies suggest that the cardiotoxicity is related to the maximum escitalopram concentration (Cmax) rather than the area under the curve (AUC) parameter. The sponsor calculated safety margins for cardiac toxicity are appropriate and presented below.

Safety Margin Calculations for Cardiotoxicity[a] of Escitalopram Based on Rat Toxicokinetic and Human Pharmacokinetic Results

Analyte		C_{max} Ratio[b]	
		Males	Females
Combined Escitalopram, S-DCT, S-DDCT	NOEL	16	24
	TOEL	31	44
Escitalopram	NOEL	8	18
	TOEL	15	31
S-DCT	NOEL	32	38
	TOEL	68	74
S-DDCT	NOEL	72	71
	TOEL	170	132

[a]For males, NOEL=40 mg/kg based on original 13-week study. Based on the 60-day bridging study, NOEL for females=80 mg/kg, TOEL for males=80 mg/kg and TOEL for females=120 mg/kg.
[b]Ratios are based C_{max} on Day 90 of the 13-week rat toxicity study (for NOEL for male rats) and Day 7 of the 60-day rat bridging study (for TOEL for male rats and NOEL/TOEL for female rats) to Day 24 interpolated C_{max} value obtained from the 20 mg escitalopram dose (mid-point values of 10 and 30 mg data assuming dose proportionality) in the multiple dose human pharmacokinetic study[46].

Figure 35, from page 53 of Nonclinical Pharmacology and Toxicology, Section 5.0 of NDA 21-323.

The Cmax, AUC and safety factors for other forms of toxicity observed in rats are presented below. The NOEL of 10 mg/kg is taken from the 13 week toxicity study with toxicokinetic parameters for the males on Day 90 of the study. The 20 mg/kg LOEL was taken from the four week toxicity study using the Day 1 toxicokinetic parameters for males. The 60 mg/kg LOEL for reduced body weight gain was taken from the same study using the Day 29 toxicokinetic parameters for males. The 80 mg/kg dose was taken from the 60 day bridging study using Day 7 toxicokinetic data for males. It was not possible to calculate ratios for Cmax and AUC in the reproductive toxicity studies, because toxicokinetic analyses were not conducted. The Cmax and AUC values were converted from nmoles/l to ng/ml by multiplying by (324 ng/nmole) X (0.001 l/ml) = 0.324. Comparisons were only made using escitalopram. No toxicokinetic data were available for the perinatal studies in rats.

Observation	Dose (mg/kg/day)	Rat/Human Ratio	Cmax (ng/ml)	Rat/Human Ratio	AUC (ng-hr/ml)	Rat/Human Ratio
Human Dose	20 mg/day[a]	---	42	---	730	---
NOEL	10	5	59	1	208	0.3
Salivation	20	10	139	3	599	0.8
NOEL for fetal effects	56	28	---	---	---	---
Reduced Body Weight Gain	60	30	406	10	2676	4
Cardiotoxicity/ Mortality	80	40	616	15	4734	6
Reduced fetal weight	112	56	---	---	---	---

[a]For calculation of Rat/Human ratio for dose, the human clinical dose (20 mg/day) was divided by 60 kg (assumed patient weight) and multiplied by 6.2 (surface area extrapolation factor for converting from humans to rats) to yield an equivalent dose of 2 mg/kg/day in the rat.

The AUC margin of safety values (ie, the ratio of rat to human exposures) are the most conservative. They range from 1 for salivaiton to only 6 for mortality and cardiotoxicity. However the cardiotoxicity appears to be more related to Cmax values in rats. In this case, the margin of safety is a more reassuring 15.

Labeling Review

The following changes to the sponsor proposed labeling are recommended. Additions to the text are indicated by underlined text, while deletions are indicated by ~~strikethrough text~~. Page and line numbers refer to the sponsors original text submitted on March 23, 2001. Many of these changes have been suggested to make the labeling consistent with that of racemic citalopram (Celexa). A problem with the development of the labeling for S-citalopram is the comparisons of pre-clinical doses of the racemic mixture with the clinical doses of S-citalopram. The effects observed in the laboratory could be due to:

1. The S-enantiomer alone.
2. An additive interaction between the R-and S-enantiomers.
3. A synergistic interaction between the R- and S-enantiomers.
4. Or, a negative interaction between the R- and S-enantiomers.

Since there is uncertainty about the proper method of comparing doses of the preclinical RS-citalopram doses and the clinical S-citalopram doses, no dose comparisons between human and experimental animals are recommended for the pre-clinical RS-citalopram studies.

Number of Pages Redacted 3

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Draft Labeling
(not releasable

RECOMMENDATIONS

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

Reviewer signature/team leader signature [Concurrence/Non-concurrence]

Paul L. Roney, Ph.D.

APPENDIX 1- SUMMARY AND EVALUATION FROM ORIGINAL REVIEW OF NDA 20-822 RS-CITALOPRAM (BY ROBIN HUFF, PH.D.)

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 1.5 - 2-fold 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.

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 1/2 - 1/3 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/m² 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/m², 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 papillae 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 11 - 12% 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/m² 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 35 - 50% of HD M and F during the latter half of the study, the increased mortality observed in HD M, and the 7 - 8% 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 0/105 - 5/400) 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 (4 - 8% relative to control) 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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/s/

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12/3/01 04:13:57 PM
PHARMACOLOGIST

Barry Rosloff
12/17/01 05:32:51 PM
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APPEARS THIS WAY
ON ORIGINAL

Pharmacology/Toxicology Review of Complete Response to Approvable Letter

NDA:	21-323
Sponsor:	Forest Laboratories, Inc.
Drug	Lexapro (escitalopram oxalate)
Indication:	[redacted]
Letter Dates:	February 20, 2002, April 10, 2002
Reviewer:	Paul Roney
Review Date	May 23, 2002

Introduction

Lexapro (escitalopram) is proposed for the treatment of Major Depressive Disorder under NDA 21-323. The Division has issued an Approvable Letter to the sponsor asking for further information on several issues, including pre-clinical issues, on January 23, 2002. The sponsor submitted a reply to Approvable Letter on February 20, 2002. In response to a FDA inquiry on March 14, 2002, further preclinical data were submitted on April 10, 2002. This report will review the Sponsor's response to the pre-clinical issues in the Approvable Letter and proposed labeling changes in the Approvable Letter.

Preclinical Issues Identified in Approvable Letter

The following text from the Approvable Letter (sent January 23, 2002) outlines the Divisions concerns about the preclinical toxicity of Lexapro:

We are concerned that the chronic toxicity studies performed in rats with racemic citalopram may not predict important toxicities that might be associated with chronic treatment with escitalopram. Our acceptance of a 13 week rat toxicity bridging study as constituting an adequate assessment of the long-term toxicity of escitalopram presupposed that the findings seen with escitalopram would be essentially similar to those seen with the racemate at appropriate doses. If this were the case, the results of the chronic toxicology studies done with the racemate could reasonably be assumed to predict the findings with chronic escitalopram use. However, in our view, this turned out not to be the case. Specifically, there was evidence of cardiac injury at the 80 mg/kg/day dose of escitalopram in both the 13 week and 60 day studies, but there was little evidence of these cardiac lesions in the racemate treated groups, including at the maximum dose of 160 mg/kg/day. These lesions did not seem to be related to higher levels of S-citalopram (or of the 2 main metabolites) in the escitalopram treated animals compared to the racemate treated animals, because the plasma levels (either Cmax or AUC) were not substantively different between the groups. Because this injury was not seen in the racemate treated animals, this raises questions about the validity of the long-term toxicity studies performed with the racemate as a predictor of the long-term toxicity of escitalopram. This may be particularly problematic in this case, because, for example, the margin of safety for S-citalopram (AUC at the NOEL of 40 mg/kg/day in the 13 week rat study, compared to the AUC of a 20 mg dose in

Line 516-520, Carcinogenesis, Mutagenesis, Impairment of Fertility, Pregnancy- Keep original FDA language.
The issue in these lines is the identification of the LOEL and NOEL for decreased pup viability (as indicated by the viability index) in the Segment 3 reproductive toxicity study. The Division proposed a LOEL of 24 mg/kg based an a statistically significant decrease in viability index. The following table presents the viability index for the control and escitalopram groups in the study.

Dose	Viability Index	Lactation Index
0 mg/kg	98.1%	98.6%
6 mg/kg	99.4%	99.4%
12 mg/kg	98.6%	97.4%
24 mg/kg	93.5%	97.8%
48 mg/kg	94.2%	93.5%

The division considers the decreased viability index at 24 mg/kg to be a drug related effect. In the response to the Approvable Letter, the sponsor argued that the 93.5% viability index was within the historical control range (91-100%), and so was not treatment related. The Division requested further information about the historical control data viability index in this laboratory. The sponsor provided this data in the April 10, 2002 submission. Viability index data were available from 54 studies conducted between July 1995 and December 2001 (the study in question was initiated in February 2000). The sponsor was concerned that data from small studies (fewer than 20 litters) would distort the statistics if given equal weight to studies with larger litter sizes. They therefore prepared an analysis of the 38 studies with 20 or more litters. An examination of the data for the two groups suggests that there are minimal differences between the two methods of evaluating the data.

	All studies (N=54)	Studies with 20+ litters (N=38)
Arithmetic Mean	97.81	97.67
Geometric Mean	97.79	97.65
Median	98.55	98.55
Mode	98.7	98.7
Standard Deviation	1.94	2.08
10^{th} Percentile	94.35	93.65
25^{th} Percentile	97.35	97.3
50^{th} Percentile	98.55	98.55
75^{th} Percentile	98.80	98.75
90^{th} Percentile	99.4	99.4
Range	91-100	91-100
Skewness	-1.73	-1.74
Kurtosis	2.68	2.42

The sponsor presents several arguments that the decrease in viability index at 24 mg/kg is not biologically significant:

1. The viability index (93.5%) is within the historical control range (91-100%).

Since this argument may give undue emphasis on studies that are outliers in the historical control database, it is not compelling. It is more appropriate to consider the historical control database as a whole. Over 90% of the studies had viability indexes greater than viability index observed at 24 mg/kg. 75% of the studies had viability indexes greater than 97.3%. The sponsor's argument also ignores the results of the study itself. The control viability index was 98.1%, which is between the 25^{th} and 50^{th} percentile of the historical control database. The two lower dose groups had viability indexes of 99.4% and 97.4%, which are also well within the historical control range. The 24 mg/kg dose group viability index is clearly lower than the concurrent control and NOEL dose groups.

2. The sponsor also argues that the 93.5% viability index is "approximately" within two standard deviations of the historical control data.

The sponsor uses the word "approximately" because the 93.5% value is outside the two standard deviation criteria when all of the studies are used. This argument assumes that the data are normally distributed. However, the data are not normally distributed. The kurtosis score of 2.68 suggests that there is a greater concentration of values around the mean than would be expected is the data were normally distributed (a kurtosis score of 0 would indicate a perfectly normal distribution). In addition, the -1.73 skewness score indicates that the distribution is skewed to the right.

3. Finally, the sponsor argues that since there was no difference in the lactation index between the control and 24 mg/kg groups, the viability index result is an aberration.

This reviewer does not consider this to be a compelling argument. A drug may the viability index for a drug without affecting the lactation index. These are separate measurement of offspring growth and development and it is not necessary for them both to be positive.

Based on these considerations, this reviewer recommends that the labeling utilize the original FDA language in this section.

Line 987-994, Animal Toxicology, Cardiovascular Changes in Rats and Dogs- Accept Forest's revision.

Recommendation

This NDA is approvable with respect to the pharmacology/toxicology portion pending labeling revision.

Reviewer signature/team leader signature [Concurrence/Non-concurrence]

Paul L. Roney, Ph.D.

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/s/

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