2. *Tox02.pdf: [June 1988] The effect of LY248686 maleate on the induction of DNA synthesis in primary cultures of adult rat hepatocytes.*

Methods: 20-hr treatment with 3H-TdR and duloxetine; autoradiography; semi-automated — counting, on 20 morphologically unaltered cells containing at least 4 grains per treatment; to highest drug concentration that did not produce "pronounced" cytotoxicity.

Results: [See Sponsor's table, below].

APPEARS THIS WAY
ON ORIGINAL

TABLE 1. THE AUTORADIOGRAPHIC IDENTIFICATION OF UNSCHEDULED DNA SYNTHESIS IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES TREATED WITH LY255485, STUDIES 870915UDS3007 AND 870922UDS3007.

Compound	Concentration Tested µg/ml	Net Nuclear Silver Grains (Mean ± SD)[a] Study 870915UDS3007	Study 870922UDS3007
LY255485[b]	1000	Toxic[c]	Toxic[c]
	500	Toxic[c]	Toxic[c]
	100	Toxic[c]	Toxic[c]
	50	Toxic[c]	Toxic[c]
	10	-1.8 ± 1.6[d]	Toxic[c]
	5	-2.5 ± 1.8	-1.9 ± 1.4
	1	-1.6 ± 2.8	-1.2 ± 1.3
	0.5	-1.2 ± 1.6	-0.9 ± 1.1
MNNG	20	30.3 ± 5.8[e]	Toxic Positive[f]
	10	17.8 ± 6.7[e]	70.3 ± 9.1[e]
	5	10.5 ± 7.3[e]	22.7 ± 10.3[e]
	1	-1.5 ± 2.2	5.9 ± 5.2[e]
2AAF	1	67.0 ± 10.4[e]	Toxic Positive[f]
	0.5	52.3 ± 10.3[e]	72.9 ± 16.5[e]
	0.1	11.8 ± 7.4[e]	37.4 ± 15.4[e]
	0.05	7.1 ± 5.1[e]	24.4 ± 8.6[e]
DMSO	1%	-2.1 ± 1.6	0.1 ± 3.0
(four replicate	1%	-2.8 ± 1.9	-2.4 ± 1.5
cultures)	1%	-2.2 ± 1.8	-1.9 ± 2.5
	1%	-1.7 ± 1.6	-1.1 ± 1.9

[a] Represents counts of nuclei from 20 morphologically unaltered cells from each treatment.
[b] LY255485 is the maleate salt of LY248686. When corrected for the potency of the free base, the doses are equivalent to 714, 357, 71.4 35.7, 7.1, 3.6, 0.7, and 0.4 ug/ml, respectively.
[c] Cytotoxic:cells unavailable for UDS.
[d] Partial toxicity, remaining unaltered cells available for evaluation.
[e] Judged to be a positive response for UDS.
[f] Cytotoxic:surviving cells positive for UDS.

Conclusion: Negative; duloxetine maleate did not induce unscheduled DNA synthesis in primary rat hepatocytes.

3. *Tox03.pdf: [June 1988] The effect of LY248686 maleate on the in vivo induction of sister chromatid exchange in bone marrow of Chinese hamsters.*

Method: Female Chinese hamsters (3/group) were treated orally with duloxetine maleate (125, 250 or 500 mg/kg, equivalent to 89.25, 178.5, or 357 mg/kg of duloxetine base, respectively; doses based upon high dose that is "~250-fold greater than the anticipated human clinical dose"), cyclophosphamide (50 mg/kg), or vehicle (10% aqueous acacia), 5 hr after sc implantation of BrdUrd ([ ] tablet; 19 hr after dosing, cells were arrested in metaphase with Velban (vinblastine); 21 hr after dosing, metaphase chromosomes were harvested from femur Inbone marrow; 100 metaphases per animals were scored for characteristic 1st, 2nd, and 3rd division staining patterns; 25 metaphases per animal were scored for SCE.

Results: Cytotoxicity (i.e., increased number of first division metaphase figures) was evident in all cyclophosphamide animals and 1/3 LD, 2/3 MD and 1/3 HD duloxetine animals and 0/3 vehicle controls.

TABLE 3. SUMMARY VALUES FOR THE IN VIVO INDUCTION OF SISTER CHROMATID EXCHANGE IN BONE MARROW OF CHINESE HAMSTERS TREATED ORALLY WITH LY255485, STUDY 871130SCB3007.

Chemical Treatment	Dose (mg/kg)	Total Metaphases Scored	SCE/Metaphase (Mean ± S.D.)	Cell Cycle (% M_1)
LY255485[a]	500	75	2.8 ± 1.7	19
LY255485[a]	250	75	2.6 ± 1.7	26[b]
LY255485[a]	125	75	3.1 ± 1.9	22[b]
Control (10% aqueous acacia)	10 ml/kg	75	2.3 ± 1.6	20
Cyclophosphamide	50	75	15.7 ± 6.8[c]	39[b]

[a] LY255485 is the maleate salt of LY248686. When corrected for the potency of the free base, the doses are equivalent to 357, 178.5, and 89.25 mg/kg LY248686, respectively.

[b] Cytotoxic response; distribution of metaphase figures shifted in favor of first division staining pattern.

[c] Judged to be a positive response for SCE induction as determined by Student's t-test ($p \leq 0.01$).

Conclusion: Negative; duloxetine maleate did not induce sister chromatid exchange in Chinese hamsters after 21-hr treatment with oral doses up to 500 mg/kg.

E. Genetic toxicology summary and conclusions: Duloxetine was adequately tested and negative in 2 out of 3 parts of the standard battery: in the Ames test for bacterial mutagenicity and the *in vivo* mouse micronucleus assay for clastogenicity. Two *in vitro* tests for clastogencity, chromosomal aberrations in CHO cells and "large" colony formation in L5178Y mouse lymphoma cells, were negative with and without metabolic activation when tested for a short duration (4 hr) of duloxetine exposure, but neither was followed up with a longer (~24 hr) treatment without activation, as recommended according to current guidelines. Additionally, each was further flawed: in the CHO cell assay, only 100 (not 200) metaphases were counted per treatment and in the mouse lymphoma assay, large and small colonies were not reported separately, although the lack of effect on total seems unlikely to mask an increase in large colonies.

Additionally, duloxetine was not genotoxic in 2 other assays: unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes and *in vivo* sister chromatid exchange in Chinese hamster bone marrow.

F. Labeling recommendations:

MUTAGENESIS: Duloxetine was not mutagenic in the *in vitro* bacterial reverse mutation assay (ames test) and was not clastogenic in an *in vivo* chromosomal aberration test in mouse bone marrow cells, [ ] Additionally, duloxetine was not genotoxic in an *in vitro* mammalian forward gene mutation assay in mouse lymphoma cells or in an *in vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes, and did not induce sister chromatid exchange in Chinese hamster bone marrow *in vivo*.

VI. CARCINOGENICITY:

A. Study title: Oncogenic and blood level studies in CD-1 mice given duloxetine hydrochloride in the diet for their life span.

Key study findings: Valid study; liver adenomas and carcinomas in female mice.

Study number: MC1113 (M01193 and M01293, replicate oncogenic studies initiated ~2 weeks apart), M01393 (blood level study).
Volume #, and page #: 1.26:1- [Tox43.pdf, Tox43d1.pdf, Tox43d2.pdf in electronic submission].
Conducting laboratory and location: Lilly Research Labs, Greenfield, IN 46140.
Date of study initiation: M01193: 7/8/93-7/13/95; M01293: 7/23/93-7/28/95; M01393: 8/2/93-1/31/95.
GLP compliance: yes, see pages 4-5.
QA report: yes, see pages 2-3.
Drug, lot #, and % purity: duloxetine hydrochloride, DPD13975, — as total enantiomers, — as the HCl salt of the enantiomers (by HPLC).
CAC concurrence: Not available at the time this study was conducted.

Study Type: 2-year bioassay.
Species/strain: CD-1 mice (~ .CDR-1 (ICR), [ ]
Number/sex/group; age at start of study: 60/sex/group; 6-7 weeks of age.
Animal housing: individually, in stainless steel cages with wire mess floors and clear plastic fronts; 12 hr light/dark, on at 0600, off at 1800 hrs; city water ad lib; — Certified Rodent Diet — ' ad lib (with or without drug).
Formulation/vehicle: in mash feed diet; prepared fresh every ~ 2 weeks.
Drug stability/homogeneity:
Methods:

Doses: 0, 0.005, 0.01, 0.03, and 0.08% in diet.
Basis of dose selection: 2-week (0, 0.025, 0.05, 0.1, 0.2% duloxetine, corresponding to 0, 40, 80, 160, and 320 mg/kg/d, as maleate salt) and 3-month (0, 0.02, 0.04, 0.08% duloxetine, as HCl salt; lot 619NK0; Tox34.pdf, studies M01692 and M01792) dietary studies.

Study reports were not submitted for the 2-week study, but results were summarized in the introduction to the longer studies. In the 2-week study, 1/10 mice died at 0.2% and body weights were decreased 9% at 0.1% dietary drug (females only) and decreased 32% at 0.2% dietary drug (presumably both males and females) compared with controls. Changes related liver function were noted: induction of P450 (CYP2B, CYP1A1) and hepatocellular hypertrophy in most dosed groups; and increased ALT and decreased cholesterol and triglycerides at 0.2% dietary drug only.

Based upon the decreased body weights at 0.2% dietary drug in the 2-week study, dietary doses of 0, 0.02, 0.04, 0.08%, as the HCl salt, were chosen for the 3-mo study. Although average body weights were only slightly lower than controls at the HD (5% for males, 3% for females after 3 mo of dosing), see figure below, the average body weight gain for this group was 10% lower (29% gain for males and 26% gain for females) than for controls (39% gain for males and 36% gain for females). The 0.08% dietary dose could be considered and MTD by current standards, based upon the 10% decrease in body weight gain in this 3-mo study.

Figure 12. Body weights in CD-1 mice treated for 3 months with duloxetine (HCl) as 0.02, 0.04, or 0.08% in diet resulting in average daily doses of 30, 60, or 131 mg/kg for males and 39, 73, or 163 mg/kg for females. [Sponsor's graph, excerpted directly from this submission; n=10/sex/group.]

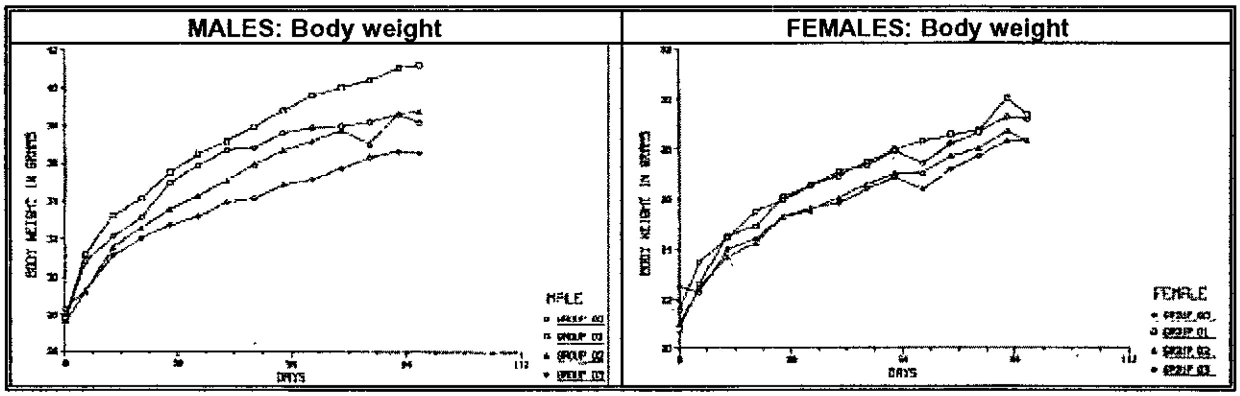
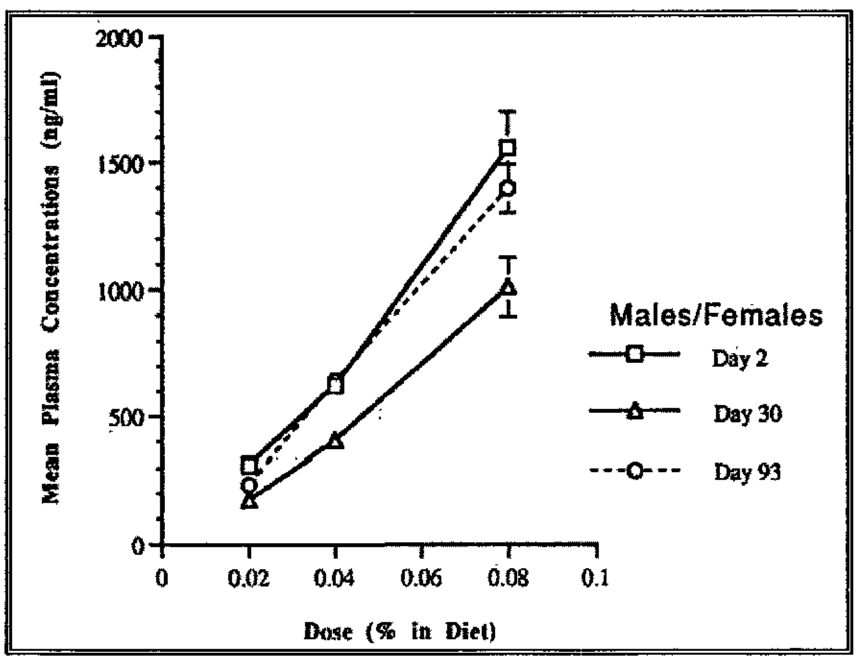


Figure 13. Plasma levels of duloxetine in CD-1 mice treated for 3 months with duloxetine (HCl) as 0.02, 0.04, or 0.08% in diet resulting in average daily doses (in main study mice) of 30, 60, or 131 mg/kg for males and 39, 73, or 163 mg/kg for females. [Sponsor's graph, excerpted directly from this submission; mean ± standard error, n=3/sex/dose/time point; blood was collected between 0730 and 0930 hrs.]



Restriction paradigm for dietary restriction studies: not restricted.
Route of administration: dietary.
Frequency of drug administration: dietary/continuous.
Dual controls employed: no.
Interim sacrifices: no.
Satellite PK study (M01393) group(s): 22/sex/dose (no negative controls); 5-6 weeks of age.
Deviations from original study protocol: on one date early in the (M01293) study (8/27/93), some mice were found in the wrong cages (#1076 in #0076's cage, #3077 in #4077's cage); the Sponsor states that this did not compromise the study, due to the short duration of the incorrect diet, and I agree.
Statistical methods: body weight and food consumption data were analyzed by sex at each time point by analysis of variance, with rack and column within rack as factors; monotonic dose-responses by sequential trend (Tukey) test; pairwise comparisons with control by Dunnett's t-test, $p<0.05$.

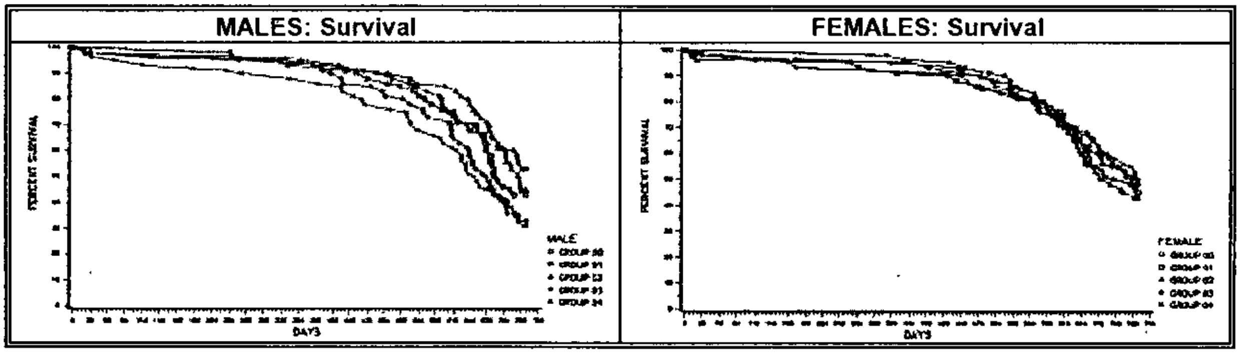
Observations and times:

Clinical signs: daily, for survival, general physical condition, and behavior; weekly detailed exam.
Body weights: weekly for first 17 weeks, then every 2 weeks.
Food consumption: weekly for first 17 weeks, then every 2 weeks.
Hematology: on surviving mice at necropsy.
Clinical chemistry: on surviving mice at necropsy.
Organ weights: see Histopathology Inventory Table 14, above.
Gross pathology: see Histopathology Inventory Table 14, above.
Histopathology: see Histopathology Inventory Table 14, above.
Toxicokinetics:
Induction of P450: EDR p195

Results:

Mortality: slight increase in mortality in HD and MHD males (see figure, below); attributed by Pathologist largely to increased incidence of mouse urologic syndrome. No clear effect in females. [#2080 was determined to be missing from the study (M01293) on 8/6/93.]

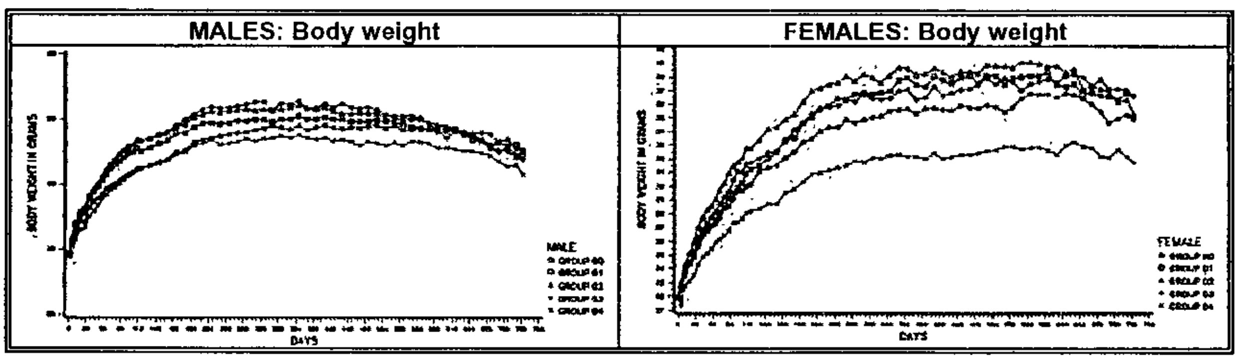
Figure 14. Survival of CD-1 mice treated for 24 months with duloxetine (HCl) as 0.005, 0.01, 0.03, or 0.08% in diet resulting in average daily doses of 6, 12, 35, or 101 mg/kg for males and 8, 15, 48, or 144 mg/kg for females. [Sponsor's graphs excerpted directly from this submission; n=60/sex/dose.]



Clinical signs: rough hair coat, scabs, and redness of the ear; and perineal soiling (in males).

Body weights: slightly decreased mean body weights in HD males for only the last half of the study (decreased 7% at termination), slightly increased body weights for other dosed males for first half of the study; decreased mean body weights in HD females throughout the study (decreased 9% at termination), but slightly (non-significantly) increased mean body weights for other dosed females.

Figure 15. Body weights in CD-1 mice treated for 24 months with duloxetine (HCl) as 0.005, 0.01, 0.03, or 0.08% in diet resulting in average daily doses of 6, 12, 35, or 101 mg/kg for males and 8, 15, 48, or 144 mg/kg for females. [Sponsor's graphs excerpted directly from this submission; starting n=60/sex/dose.]



Food consumption: statistical analysis on cumulative daily food consumption (g/mouse and g/kg body weight) and cumulative EFU (i.e., efficiency of food utilization, kg body weight gain/g food consumed), only; not on absolute daily food consumption. Apparent cumulative food consumption was slightly decreased in HD females throughout most of the study, however, body weights in this group were lower than would have been predicted from the apparent decrease in food consumed (i.e., HDF gained less weight per amount of food apparently consumed).

Hematology: No remarkable findings. Only on surviving mice at termination of study: minimal (<10%) decreases of some RBC-related parameters in HD males; slight (12%) increase in platelet count in HD females.

Clinical chemistry: No remarkable findings. Only on surviving mice at termination of study: dose-related decreases in total bilirubin in males and females at doses ≥ 0.01%, up to ~30% decrease at 0.08% dose; increases in ALP, ALT, AST in some HD males and in mean ALP (~50%↑) and AST (2-fold↑) for HD females; slight (6%) increase in creatinine in HD females.

Organ weights: decreased body weights at HD (HFM ↓7%, HDF ↓9%, vs controls, at terminal sacrifice); increased (absolute and relative) liver weights in HD males and females and HMDF and in LMDF (relative, only); decreased (relative) spleen weight in HDM; increased (absolute) kidney weights in HD males and females.

Gross pathology: The Pathologist attributed soiling and penis distension to mouse urologic syndrome.

Table 20. Incidence of macroscopic observations with treatment relationships in CD-1 mice treated for 2 years with dietary duloxetine. [Pathologist's table excerpted directly.]

	Group 00		Group 01		Group 02		Group 03		Group 04	
Observations	M	F	M	F	M	F	M	F	M	F
Whole Animal										
Soiled	13	6	13	9	18	9	21	9	27	8
Kidney										
Cyst	22	8	24	4	30	6	15	7	10	2
Liver										
Cyst, biliary	0	0	0	1	0	1	2	3	6	2
Nodule	18	2	22	4	23	10	27	9	18	17
Penis										
Distension	0		2		4		3		4	

Histopathology:

Non-neoplastic: liver (see table, below); heart (slightly increased incidence of [minimal to slight] myocardial degeneration) in males at 3 higher doses; and urinary tract (mineralized debris in renal tubules, hyperdistension of bladder, penile distension and inflammation, considered manifestations of MUS) in males.

Table 21. Sponsor's table showing incidence of compound-related histopathologic changes in liver tissues.

	Group 00		Group 01		Group 02		Group 03		Group 04	
Lesion	M	F	M	F	M	F	M	F	M	F
No. Tissues Examined	60	60	60	60	60	59	60	60	60	60
Hepatocellular Hypertrophy	10	3	12	1	22	9	47	26	49	55
Hepatocellular Vacuolation	6	7	16	9	10	15	7	22	9	15
Single Cell Necrosis	4	2	4	2	2	0	1	6	5	5
Hepatocellular Adenoma	10	2	13	1	13	4	19	4	10	13
Hepatocellular Carcinoma	11	0	10	0	12	2	8	2	14	6

Neoplastic: In females only: Increased incidence of hepatocellular adenomas and carcinomas and of benign endometrial stromal tumors. The incidence of benign endometrial stromal tumors appeared to be increased in a dose-dependent manner, especially at the HD, where the incidence was 4/60 (6.7%) compared with 1/60 (1.7%) in controls (see Sponsor's table, below). However, the incidence even at the HD was quite low and well with in the range of historical incidence for endometrial stromal polyps, from 1.7-17% in 35 studies reported by Charles Rivers' (March 2000).

APPEARS THIS WAY
ON ORIGINAL

Table 22. Sponsor's table of tumor incidence and further analyses on tumors with significant increasing trends based upon the normal approximation p-values.

	Treatment Group 00	01	02	03	04		
Females: Liver - Malignant Hepatocellular Carcinoma	0	0	2	2	6		
						z	one-sided p-value
	Increasing Trend in Groups 00 up to 04					3.029	0.001
	Increasing Trend in Groups 00 up to 03					1.760	0.062[a]
Females: Liver - Benign Hepatocellular Adenoma	2	1	4	4	13		
						z	one-sided p-value
	Increasing Trend in Groups 00 up to 04					3.738	<0.001
	Increasing Trend in Groups 00 up to 03					1.255	0.105
Females: Uterus - Benign Endometrial Stromal Tumor	1	1	0	2	4		
						z	one-sided p-value
	Increasing Trend in Groups 00 up to 04					0.422	0.048
	Increasing Trend in Groups 00 up to 04					0.422	0.062[a]

[a]Exact one-sided p-value from randomization trend test.

The Sponsor found increased incidences of **benign hepatocellular adenomas and malignant hepatocellular carcinomas in females**, especially at the HD (see Sponsor's table above). In the table below, I have tabulated the incidence of these neoplasias, separately and combined, for males as well as females, along with the historical ranges reported by Charles Rivers (March 2000). There is a clear increase in incidence of both adenomas and carcinomas in HD female mice, with the incidence of adenoma and/or carcinoma increased nearly 10-fold compared to control. The incidence of adenomas increased from ~3% (10/60) in control females to 22% (13/59) for HD females; approximately equal to that in males (treated or not). The incidence of carcinomas increased from 0% (0/60) for control females to 10% (6/60) for HD females; this was still only approximately half that in males (treated or not).

Table 23. Incidence of hepatocellular adenomas and carcinomas in CD-1 mice treated with dietary duloxetine for 2 years. Number of livers examined was 59-60 per group. [I checked individual histopathology reports for this data: 1 mouse (0.01% female) was completely missing, livers from 3 other mice (0.005% female, 0.03% male, 0.08% female) were autolyzed.] [Historical controls are ranges from studies (~40 for males, ~15 for females) presented in Charles Rivers' March 2000 report.]

Hepatocellular lesion	Sex	control	0.005%	0.01%	0.03%	0.08%	historicals
Adenoma	M	10	13	13	19 (32%)	10 (17%)	2.9-28%
	F	2	1	4	4	13 (22%)	0.85-7.8%
Carcinoma	M	11	10	12	8	14 (23%)	1.5-16%
	F	0	0	2	2	6 (10%)	1.4-4.3%
Adenoma and/or carcinoma	M	18	21	23	25	23	N/a
	F	2	1	6	5	18	N/a

Because histiocytic sarcomas were found in HD female rats, I looked at the incidence of this whole body tumor in the mice in this study (see table, below). There is no evidence of treatment-related increased incidence of this tumor in male mice and the values are within the historical control range. There was considerable variability in the incidence of this tumor among female treatment groups, although all fell within historical control ranges and there was no simple dose-response relationship. [This tumor appears to occur more frequently in CD-1 mice than in Fischer 344 rats.]

Table 24. Incidence of whole body histiocytic sarcomas in CD-1 mice (60/sex/group) treated with dietary duloxetine for 2 years. [Historical controls are ranges from studies (19 for males, 31 for females) presented in Charles Rivers' March 2000 report.]

Tumor	Sex	control	0.005%	0.01%	0.03%	0.08%	historicals
Histiocytic sarcoma	M	2	3	1	2	0	1.1-8.0%
(whole body)	F	1	6	5	6	1	1.7-18%

Peer review: of 5/sex/group at control and HD, with special attention to liver and hyperplastic lesions in all tissues; and lesions in all mice diagnosed by the primary pathologist as neoplastic were examined microscopically. Quoting from the peer review certification:

"

There were no differences in pathologic interpretation with the primary pathologist relative to treatment-related lesions, which consisted of hepatocellular hypertrophy and vacuolation in males and females treated with doses at or greater than 0.01 mg/kg and hepatocellular adenomas and carcinomas in females treated with 0.08 mg/kg.

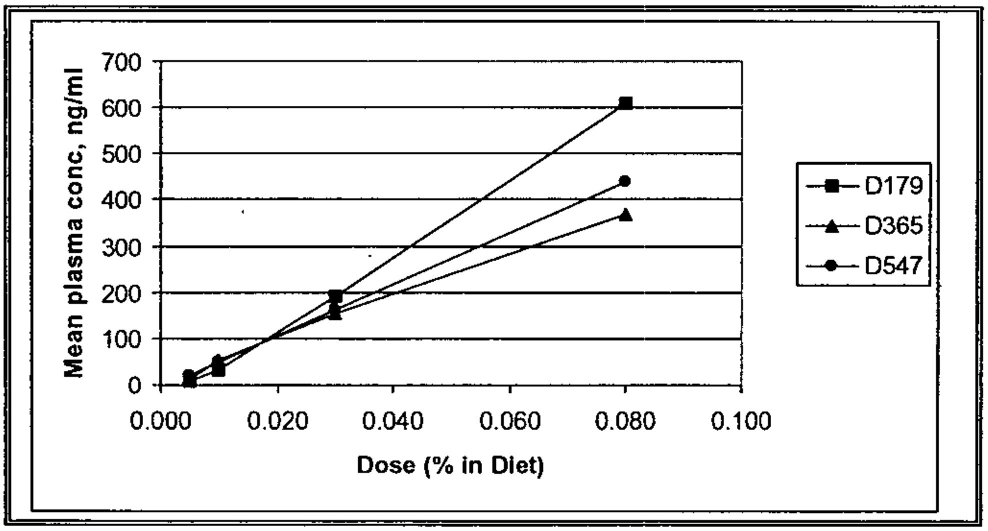
In summary, there were no substantive differences in the histopathology findings or interpretation of morphologic pathology data as described by the study pathologist and the reviewing pathologist. It is my independent opinion that the original pathologic evaluation of Studies M01193 and M01293 is complete and accurate.

"

APPEARS THIS WAY
ON ORIGINAL

Toxicokinetics:

Figure 16. Plasma levels of duloxetine in CD-1 mice treated for 18 months with duloxetine (HCl) as 0.005, 0.01, 0.03, or 0.08% in diet resulting in average daily doses (in main study mice) of 6, 12, 35, or 101 mg/kg for males and 8, 15, 48, or 144 mg/kg for females. [Graph of Sponsor's summary data averaged across sexes; mean, n=3/sex/dose/time point; blood was collected between 0730 and 0930 hrs.]



Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The HD was adequate, based upon decreased body weight gain in the 3-mo dose-ranging study and upon decreased body weights in both males and females and slightly increased mortality in males in this study.

Evaluation of tumor findings: Increased incidence of tumors was limited to benign endometrial stromal tumors in the uteruses and hepatocellular adenomas and carcinomas in the livers of HD females, only.

B. Study title: A chronic/oncogenic study in Fischer 344 rats given duloxetine hydrochloride in the diet for 2 years.

Key study findings: Valid; no treatment-related tumor findings.

Study number: R03893 and R03993 (RC0389).
Volume #, and page #: 1.29-1.30 [Tox44.pdf, Tox44d1.pdf, Tox44d2.pdf in electronic submission].
Conducting laboratory and location: Lilly Research Labs, Greenfield, IN 46140.
Date of study initiation: performed in 2 phases, 2 weeks apart, with dosing from 3/3/93-3/9/95 for one phase (R03893) and from 3/18/93-3/24/95 for the other (R03993).
GLP compliance: yes, see page 4 of Tox44.pdf.
QA report: yes, see pages 2-3 of Tox44.pdf.
Drug, lot #, and % purity: duloxetine hydrochloride, lot # DPD13975, — duloxetine base, XXX% pure.
CAC concurrence: Not available at the time this study was conducted.

Study Type: 2 yr bioassay.
Species/strain: Fisher 344 rats ( ]
Number/sex/group: 60/sex/group.
Age at start of study: 6-9 weeks (males, 120.1±6.6 g; females, 96.3±4.9 g).
Animal housing: individually in ventilated racks; . Certified Rodent Chow
Formulation/vehicle: in mash feed diet; prepared fresh every ~ 2 weeks.
Drug stability/homogeneity:
Methods:

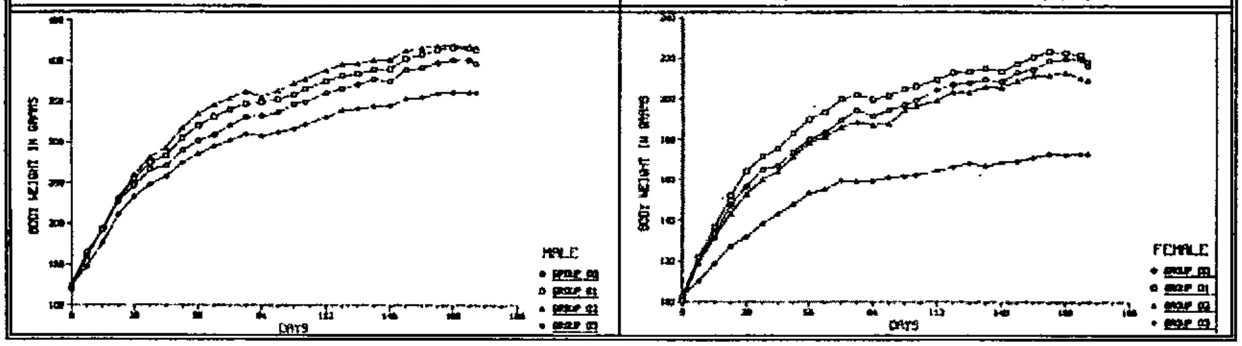
Doses: dietary levels of 0, 0.01%, 0.02%, and 0.05% (females only) or 0.08% (males only) provided average daily doses of 0, 4.37, 8.50, and 35.8 mg/kg for males and 0, 5.43, 10.6, and 26.7 mg/kg for females.

Basis of dose selection: subchronic (1-mo and 3-mo, Tox05.pdf), chronic (6-mo, Tox31.pdf) and pharmacokinetic studies:

1) ↓ body weight gain (vs controls) after 0.08% dose for 3 mo: essentially identical results in 3-mo study and at 3 mo in 6-mo study, ↓ ~12% in males and ~35% in females;
2) ↓ body weight gain (↓11% vs controls) after 0.03% dose for 3 mo in females only;
3) ↓ body weight (vs controls) after 0.08% dose in 6-mo study: ↓9% in males, ↓20% in females (see graphs below).

Figure 17. Dietary duloxetine (0.08%, Group 03 in graphs below) decreased body weights in male and female Fischer 344 rats in a 6-mo study. Dietary doses were 0% (group 00), 0.005% (group 01), 0.02% (group 02), and 0.08% (group 03). [Graphs were excerpted directly from Sponsor's submission.]

MALE FISCHER 344 RATS	FEMALE FISCHER 344 RATS



Restriction paradigm for dietary restriction studies: *ad libitum* food.
Route of administration: dietary.
Frequency of drug administration: dietary/continuous.
Dual controls employed: No.
Interim sacrifices: No.
Satellite PK or special study group(s):
Deviations from original study protocol: nothing substantial.
Statistical methods: Survival curves were compared by method of Tarone (1975). Peto's survival-adjusted trend test (Peto et al., 1980) was used as a screen to identify individual sites/neoplasms of potential concern. Quoting from the Sponsor, "all findings which resulted in one-tailed p-values less than or equal to 0.05 are documented and discussed."

Observations and times:

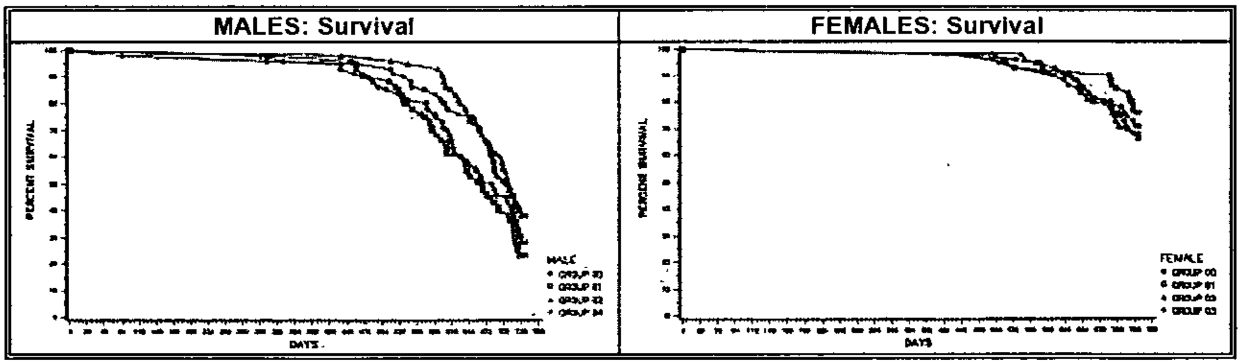
Clinical signs: daily for dead and moribund rats.
Body weights: weekly (Tuesdays) and at necropsy.
Food consumption: weekly (Tuesdays) and at necropsy.
Hematology: at ~6, 12, 18, and 24 mo; blood from orbital plexus, under isoflurane; 10/sex/group/time; RBC count, Hb, PCV, MCV, MCH, MCHC, total and differential WBC count, platelets count.
Clinical chemistry: at ~6, 12, 18, and 24 mo; blood from orbital plexus, under isoflurane; 10/sex/group/time; glucose, BUN, creatinine, total bilirubin, ALP, ALT, AST, GGT, CPK, Ca, PPi, Na, K, Cl, cholesterol, TAGs, total protein, albumin, globulin, A/G ratio.
Urinalysis: at ~6, 12, 18, and 24 mo; 10/sex/dose/time.
Organ weights: see Histopathology Inventory Table 14, above.
Gross pathology: see Histopathology Inventory Table 14, above.
Histopathology: see Histopathology Inventory Table 14, above.
Toxicokinetics: 3/sex/dose; days 181, 357, and 546 (i.e., months 6, 12, and 18).

Hepatic Enzyme Induction: benzphetamine N-demethylase; erythromycin N-demethylase; 7-ethoxyresorufin O-deethylase; P-450 content.

Results:

Mortality: Survival at the end of the 2-year study was not affected by treatment. A total of 233 rats, 163 males and 70 females, died or were killed moribund before the 2-year termination. The Pathologist identified progressive glomerulonephrosis (66% of males, 34% of females), mononuclear cell leukemia (44% of males, 34% of females), and pituitary neoplasia (adenoma or adenocarcinoma; 24% of males, 34% of females) as the 3 most common fatal conditions (causes of death or moribundity), with more than 1 fatal lesion not uncommon. Of these conditions, only pituitary neoplasias were affected by duloxetine treatment (see neoplastic findings, below).

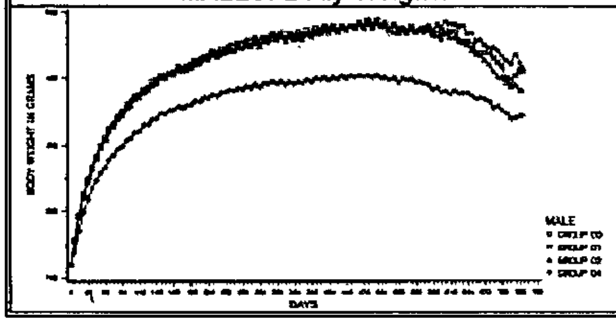
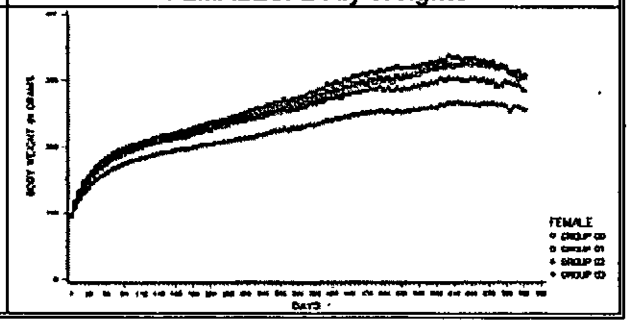
Figure 18. Survival of Fisher 344 treated for 24 months with duloxetine (HCl) at dietary levels of 0, 0.01%, 0.02%, and 0.05% (females only) or 0.08% (males only), providing average daily doses of 0, 4.37, 8.50, and 35.8 mg/kg for males and 0, 5.43, 10.6, and 26.7 mg/kg for females. [Sponsor's graphs excerpted directly from this submission; n=60/sex/dose.]



Clinical signs: Sponsor noted increased chromorhinorrhea and scabs and ulcerations of the hindpaw in high dose males; increased lacrimation in all treated females and increased incidence of soiling in MD and HD females.

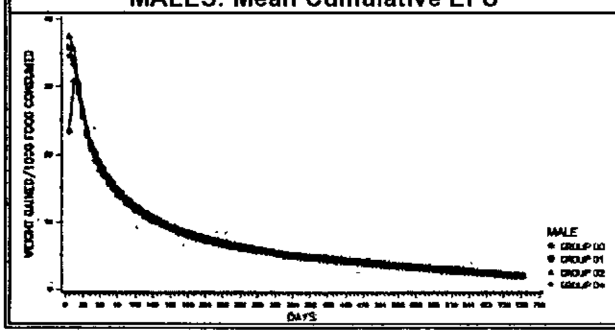
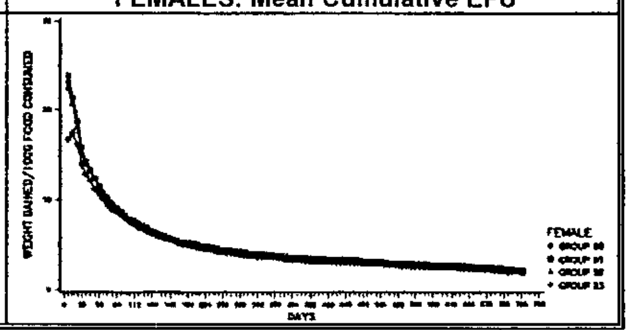
Body weights: High dose male rats weighed less than controls from ~week 8 through the end of the study; at week 13 body weight gains were 21% less than controls; at 6, 12, 18 and 24 mo, body weights were 13, 15, 17, and 17%, respectively, less than controls. A similar pattern was seen in high-dose females: at week 13 body weight gains were 24% less than controls; at 6, 12, 18 and 24 mo, body weights were 12, 16, 21, and 17%, respectively, less than controls.

Figure 19. Body weights in Fisher 344 treated for 24 months with duloxetine (HCl) at dietary levels of 0, 0.01%, 0.02%, and 0.05% (females only) or 0.08% (males only), providing average daily doses of 0, 4.37, 8.50, and 35.8 mg/kg for males and 0, 5.43, 10.6, and 26.7 mg/kg for females. [Sponsor's graphs excerpted directly from this submission; n=60/sex/dose.]

MALES: Body Weights	FEMALES: Body Weights
	

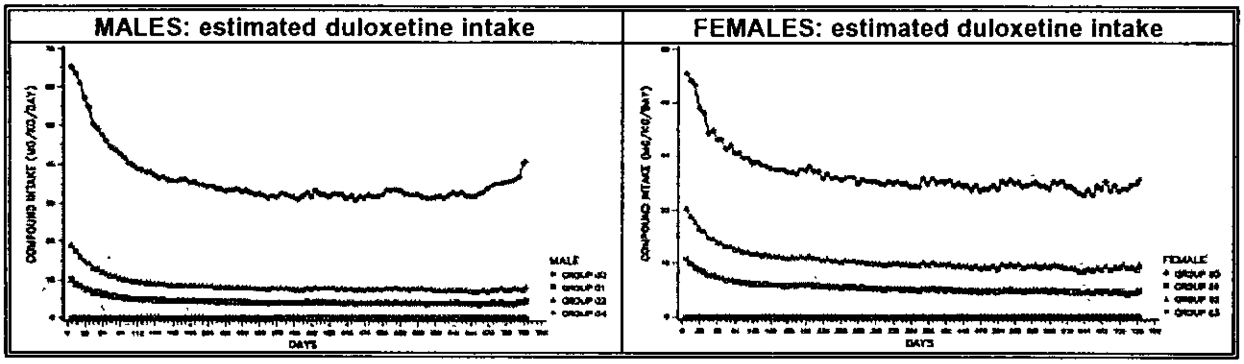
Food consumption: EFU was decreased for HD males and females for the first week of dosing only (see Sponsor's graphs, below).

Figure 20. Cumulative efficiency of food utilization (EFU, based upon estimated food consumption) in Fisher 344 treated for 24 months with duloxetine (HCl) at dietary levels of 0, 0.01%, 0.02%, and 0.05% (females only) or 0.08% (males only), providing average daily doses of 0, 4.37, 8.50, and 35.8 mg/kg for males and 0, 5.43, 10.6, and 26.7 mg/kg for females. [Sponsor's graphs excerpted directly from this submission; n=60/sex/dose.]

MALES: Mean Cumulative EFU	FEMALES: Mean Cumulative EFU
	

Estimated drug doses: Drug doses, mg/kg/d, based upon food consumption, decreased during the initial weeks of dosing, but were quite stable from ~3 mo to the end of the study (see figure, below). Average doses were estimated as 0, 4.4, 8.5, and 36 mg/kg/d for males and 0, 5.4, 11, and 27 mg/kg/d for females.

Figure 21. Estimated duloxetine consumption (based upon estimated food consumption) in Fisher 344 treated for 24 months with duloxetine (HCl) at dietary levels of 0, 0.01%, 0.02%, and 0.05% (females only) or 0.08% (males only). [Sponsor's graphs excerpted directly from this submission; n=60/sex/dose.]



Hematology: No important changes attributable to treatment; slight (<10%) decreases in RBC-related parameters in HDM throughout the study, consistent with slight anemia.

Clinical chemistry: No important changes attributable to treatment; decreases in cholesterol, triglycerides, and creatine kinase at HD, consistent with decreased body weights.

Organ weights: Weights of several organs were decreased in the HD groups, however, only thyroid in HD males and females and prostate in HD males weighed significantly less than controls when corrected for the decrease (~17%) in body weights in these groups (i.e., when normalized to brain weight or body weight). There were no gross or microscopic changes associated with the low thyroid weight, however, the low prostate weights correlated with small size noted at necropsy and microscopic observations of subacute and chronic inflammation.

Histopathology: Peer review was done on all tissues from 10/sex for control and HD groups; additionally, the sections of liver and all neoplastic and hyperplastic lesions were examined microscopically. No substantive differences were found.

Non-neoplastic: The Pathologist noted only 2 treatment related changes (other than pituitary neoplasias): 1) increased occurrence of cholesterol granuloma in liver of males from MD and HD groups; and 2) dose-related increase in multi-nucleated (3-40) hepatocytes (MNHs), with more females affected than males.

Table 25. Incidence (out of 60/sex/group) of non-neoplastic findings in liver hepatocytes.

HEPATOCYTE FINDING	SEX	CONTROL	0.01%	0.02%	0.05%	0.08%
Liver cholesterol granulomas	M	7	9	20	-	49
	F	28	29	25	38	-
Multi-nucleated hepatocytes: total	M	1	2	11	-	20
	F	11	24	38	48	-

HEPATOCYTE FINDING	SEX	CONTROL	0.01%	0.02%	0.05%	0.08%
Minimal MNHs	M	1	1	6	-	19
	F	12	18	27	22	-
Slight MNHs	M	0	1	4	-	1
	F	0	6	11	21	-
Moderate MNHs	M	0	0	1	-	0
	F	0	0	0	5	-

Neoplastic: Pituitary neoplasia (especially adenoma) was common in this study (see table, below). Duloxetine treatment decreased the incidence of pituitary adenomas in males, especially at the HD of 0.08%, where there were only 14 rats affected, compared with 40 in the control group. The fraction of these pituitary tumors that were fatal did not appear to be influenced by treatment (see Table 26, below).

Table 26. Decreased incidence (out of 60/sex/group) of neoplastic findings in pituitary of male Fischer 344 rats treated with dietary duloxetine.

PITUITARY FINDING	SEX	CONTROL	0.01%	0.02%	0.05%	0.08%
Adenomas	M	40	24	28	-	14
	F	34	32	31	23	-
Adenocarcinomas	M	0	0	0	-	0
	F	2	0	4	1	-
Total/combined neoplasias	M	40	24	28	-	14
	F	36	32	35	24	-
Fatal neoplasias	M	14	13	8	-	5
	F	10	4	5	5	-
As % of total neoplasias	M	35%	54%	29%	-	36%
	F	28%	12%	14%	21%	-

The Sponsor found that the only suggestive statistically significant increasing trend (p=0.013) was seen for **testicular interstitial cell tumor** in HDM, but argued (persuasively, I think) that "...a single p-value of 0.013 from a very common benign tumor, testicular interstitial cell tumor, alone would not provide sufficient evidence to conclude that LY248686 [duloxetine] hydrochloride is carcinogenic." Including the incidence of hyperplasia (see Table 27, below) does not appreciably alter this conclusion.

Table 27. Slightly increased incidence (out of 60/group) of interstitial cell tumor in testes of male Fischer 344 rats treated with dietary duloxetine.

INTERSTITIAL CELL FINDING	SEX	CONTROL	0.01%	0.02%	0.08%
Hyperplasia	M	6	12	7	9
Tumor (benign)	M	35	35	40	42
Hyperplasia/tumor	M	37	47	47	51

Histiocytic sarcoma (whole animal) was found in 2/60 HDF, but was found in no other groups. Of these, one rat (study R03993, #3078) was killed moribund after 449 days of dosing (cause of death: histiocytic sarcoma from mesentery LN; and moderate hepatitis), the other (study R03993, #3073) died after 726 days of dosing (cause of death: histiocytic sarcoma from LN adjacent to left adrenal; and severe hepatitis); both had metasteses in several organs, including liver and lung, in addition to the lymph node of origin.

The Sponsor's statistical analysis initially found this significant (p=0.030), but "since there were only 2 females diagnosed with whole animal histiocytic sarcoma, the randomization test was performed and a non-significant p-value of 0.065 was obtained." With this lack of statistical significance, the Sponsor dropped further mention of this tumor.

From my perspective, the finding of 2 occurrences of histiocytic sarcoma would not be of concern, except that they occurred in a high-dose group and this is a very rare tumor in Fischer 344 rats. Whereas 2/60 HD female rats had this malignant tumor, there were no other occurrences in this study (0/420 rats, 240 males, 180 females). Historical control values were not supplied with this submission and I did not find historical control values on the ' —— web site (the supplier of the Fischer 344 rats used here). However, web sites for both NTP and Charles River Labs did have information on Fischer 344 rats: NTP reported total incidence of 3/1004 males and 2/1001 females (20 studies; updated 12/99); CRL reported total incidence of 4/846 males and 1/846 females (10 studies; February 1990).

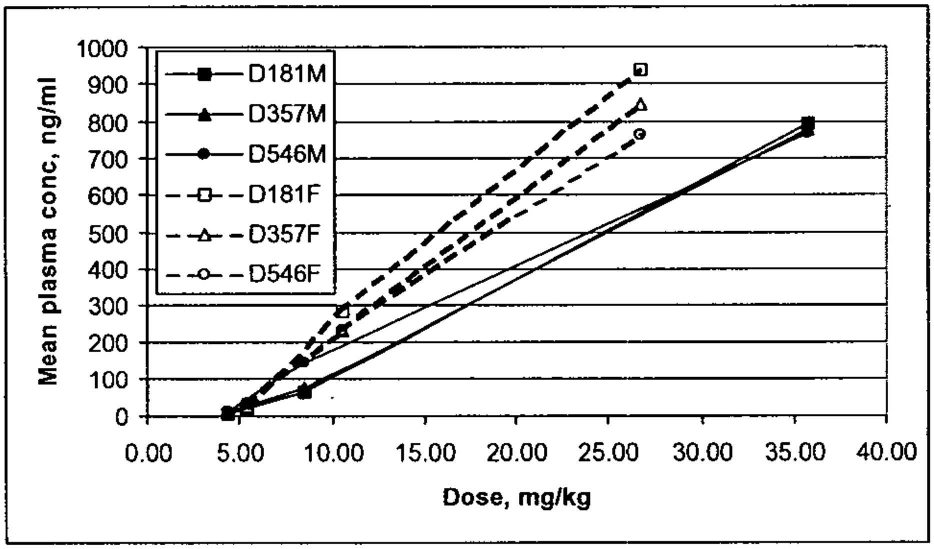
In contrast to the mouse study findings, hepatocellular adenomas and carcinomas were not common in either sex of rats and were not (clearly) more common in drug-treated rats (see table below).

Table 28. Incidence (out of 60/sex/group) of neoplastic and (possibly) pre-neoplastic findings in liver hepatocytes in Fischer 344 rats.

HEPATOCYTE FINDING	SEX	CONTROL	0.01%	0.02%	0.05%	0.08%
Hyperplasia	M	3	3	4	-	2
	F	6	6	3	2	-
Adenoma	M	1	0	1	-	0
	F	1	1	1	2	-
Carcinoma	M	0	1	0	-	0
	F	0	0	0	1	-
Adenoma/carcinoma	M	1	1	1	-	0
	F	1	1	1	3	-

Toxicokinetics: Plasma concentrations of duloxetine measured on days 181, 357, and 546 (i.e., ~6, 12, and 18 mo) in the same 3 rats/sex/group on all days. Plasma levels were linear with dose for both males and females and there was no difference in the curves from different durations of dosing. However, there was a sex-related difference: plasma levels appeared to be higher in females than males at equivalent doses (on a mg/kg/basis) at the high doses (see figure, below).

Figure 22. Plasma levels of duloxetine in male (solid symbols) and female (open symbols) Fisher 344 rats treated for 181, 357, or 546 days with duloxetine (HCl) at dietary levels of 0, 0.01%, 0.02%, and 0.05% (females only) or 0.08% (males only), providing average daily doses of 0, 4.37, 8.50, and 35.8 mg/kg for males and 0, 5.43, 10.6, and 26.7 mg/kg for females. [Graph of Sponsor's summary data; mean, n=3/sex/dose/time point; blood was collected between 0730 and 0930 hrs.]



Liver Enzyme Induction: increases in EROD, BND, and END activities at HD for males and females, indicating induction of CYP1A, 2B, and 3A, respectively. Total P450 activity was not affected, presumably because the increases for most individual isoforms were modest (see Sponsor's table, below).

Table 29. Statistically significant increases (i.e., induction) in *in vitro* liver enzyme activities [Excerpted directly from this submission].

Treatment and Sex	EROD	BND	END	P450
0.01% Male				
0.02% Male				
0.08% Male	+328%	+58%		
0.01% Female				
0.02% Female				
0.05% Female	+245%	+49%	+37%	

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The HD was adequate, based upon decreased body weight gain in the 3-mo dose-ranging study and upon decreased body weights (15-20%) in both males and females in this study.

Evaluation of tumor findings: Tumor findings were limited to 1) occurrence of histiocytic sarcoma in 2/60 HD female rats, but no other rats; and 2) a slight increase in the incidence of benign interstitial cell tumor in testes of male rats, a common, benign tumor.

C. Carcinogenicity summary and conclusions:

At the 7/30/02 meeting (meeting minutes appended to this review), the Executive Carcinogencity Assessment Committee agreed with the Sponsor and the Reviewer that the mouse study was adequate and that the positive carcinogenicity findings were hepatocellular adenomas and carcinomas in livers of high-dose females (~140 mg/kg) and no findings in high-dose males (at ~100 mg/kg).

The Committee agreed that the rat study was negative for carcinogenicity findings. The Committee felt that the rat study would be considered adequate, if it could be determined that the decreased body weights (and food consumption), which limited the doses, might be due to pharmacologically-mediated appetite suppression rather than diminished palatability. [The evidence (found after the meeting and described in the Meeting Minutes) that administration by oral gavage or intraperitoneal injection also decreased body weights and food consumption should alleviate this concern.]

F. Carcinogenicity Labeling Recommendations:

Duloxetine was administered in the diet to mice and rats for 2 years. In female mice receiving duloxetine at dietary doses of approximately 140 mg/kg/day — times the maximum recommenede human dose [MRHD] on a mg/m^2 basis), there was an increased incidence of hepatocellular adenomas and carcinomas; the no-effect level was approximately 50 mg/kg — times the MRHD on a mg/m^2 basis). Tumor incidence was not increased in male mice receiving duloxetine at dietary doses up to approximately 100 mg/kg/day — times the MRHD on a mg/m^2 basis).

In rats, dietary doses of duloxetine up to approximately 27 mg/kg/day in females — times the MRHD on a mg/m^2 basis) or approximately 36 mg/kg/day in males — times the MRHD on a mg/m^2 basis) did not increase the incidence of tumors.

APPEARS THIS WAY
ON ORIGINAL

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

A. Rat fertility (Segment I) studies: male and female rats treated in separate studies.

1. *Study title: A 14-week male fertility study of LY248686 [duloxetine] hydrochloride administered by oral gavage to CD rats.*

Key study findings:

- Decreased body weight, body weight gain and food consumption at HD (45 mg/kg);
- Decreased fertility index in MD (10 mg/kg) males only;
- Increased early resorptions and post-implantation losses in females mated with MD males only.

Study no.: R15490.
Volume #, and page #: EDR file Tox25.pdf; 120 pages.
Conducting laboratory and location: Lilly Research Labs, Greenfield, IN.
Date of study initiation: October 29, 1990, terminating February 6, 1991.
GLP compliance: yes, see page 3.
QA reports: yes, see page 2.
Drug, lot #, and % purity: duloxetine hydrochloride; lot no. 508NKO; — , (+) enantiomer free base.
Formulation/vehicle: oral gavage of suspension in 10% aqueous acacia solution (with simethicone emulsion added to reduce foaming); 5 ml/kg.

Methods:

Species/strain: CD rats — CD (SD), ⌊ ⌉. 5 wk old males, weighing ~170 g and ~8-wk old adult virgin females, weighting ~210 g.
Doses employed: 0, 2, 10, 45 mg/kg/d of duloxetine.
Route of administration: oral gavage, 5 ml/kg.
Study design: females were untreated; males were treated for 14 weeks total, 10-wk pre-mating followed by 4-wk reproduction period; cohabitation was for 1 wk or until mating was confirmed by copulatory plugs and sperm in vaginal lavage; males that did not mate during first round were cohabited with a different female for an additional wk.
Number/sex/group: 20 males/group; at least 20 females/group.
Parameters and endpoints evaluated: survival and clinical signs daily, body weight, food consumption, and EFU weekly, mating performance and fertility, reproduction (implantations, pre- and post implantation losses), sperm motility, testes weight.

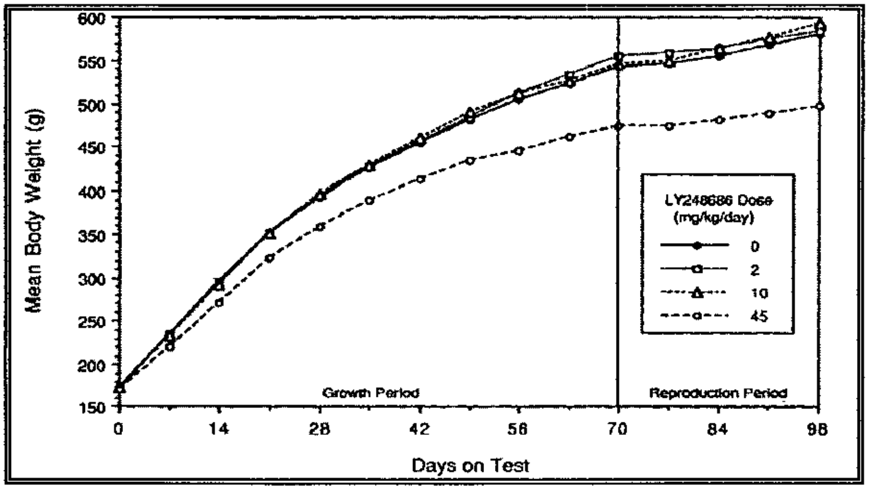
Results:

Mortality: none.

Clinical signs: intermittent salivation in all males at MD and HD; usually ~10 min after dosing.

Body weight and food consumption: decreased body weight, body weight gain and food consumption at HD. Body weight gains for HD males were decreased (↓20%, vs controls) after 7 days of dosing (the earliest time measured) and this decrease persisted to the end of the study (see table, below). HD males gained 20% less weight than controls in the 14-wk study and ate ~20% less food than controls throughout the study, with no apparent effect on efficiency of food utilization (EFU).

Figure 23. Duloxetine, administered as the hydrochloride salt by oral gavage, decreased body weight gain in young male rats. [Sponsor's graph excerpted directly from this submission.]



Toxicokinetics: not performed in this study.

Male mating performance: Duloxetine did not alter the mating index or time to mating (precoital period) (see Table 30, below); essentially all male rats, regardless of treatment, mated with untreated females, most in first pairing, after an average of ~3 days of pairing. The fertility index was 95-100% for controls, LD and HD males; essentially all matings resulted in pregnancies in these groups. The fertility index was significantly lower for MD males, only ~80%; 4 of the 19 MD males that mated did not produce pregnancies (see Table 30, below). These 4 MD males all mated in first pairings, with precoital periods of 1 or 3 days (2 males each). There was no further information provided in this study that would support or help explain the apparent decreased fertility of MD, but not HD, males. [NB Hypospermatogenesis was noted for 2/10 male rats (at each dose) treated for 6 mo with dietary doses equivalent to ~10 and ~50 mg/kg/d.]

Reproductive parameters for females from fertile matings showed no effect of treatment on average number of corpora lutea or implantations or % pre-implantation losses per dam. However, there were significantly increased dead implantations/early resorptions at LD and MD (~twice the number in control litters) that were reflected in increased post-implantation losses at MD (see Table 31, below).

Table 30. Sponsor's tables of mating and fertility indices (left panel) and time to mating (precoital period, right panel) for male rats administered duloxetine as the HCl salt by oral gavage. [Excerpted directly from this submission.]

LY248686 DOSE (MG/KG/DAY)	STATISTIC	MATING INDEX(a)	FERTILITY INDEX(b)
0	COUNT	20	20
	NO. OBS	20	20
	PERCENT	100.00	100.00
2	COUNT	20	19
	NO. OBS	20	20
	PERCENT	100.00	95.00
10	COUNT	19	15
	NO. OBS	20	19
	PERCENT	95.00	78.95*
45	COUNT	20	19
	NO. OBS	20	20
	PERCENT	100.00	95.00

* : P <= .05, TWO TAILED FREEMAN-TUKEY, BONFERRONI Z. PER COMPARISON ERROR RATE = .0083333(VARIABLE).

LY248686 DOSE (MG/KG/DAY)	STATISTIC	PRECOITAL PERIOD (DAYS)
0	MEAN	2.7
	STD	1.2
	STDERR	0.3
	N	20
2	MEAN	3.0
	STD	1.5
	STDERR	0.3
	N	20
10	MEAN	2.3
	STD	1.1
	STDERR	0.2
	N	19
45	MEAN	2.7
	STD	0.9
	STDERR	0.2
	N	20

* : P <= .05, TWO TAILED DUNNETT T ON RAW DATA.
# : P <= .001, BARTLETT TEST FOR VARIABILITY.

Table 31. Sponsor's table of reproduction parameters for untreated female rats mated to male rats administered duloxetine as the HCl salt by oral gavage. [Excerpted directly from this submission.]

LY248686 DOSE (MG/KG/DAY)	STATISTIC	CORPORA LUTEA	IMPLANTATIONS	DEAD IMPLANTATIONS(a)	PREIMPLANTATION LOSS(b)	POSTIMPLANTATION LOSS(c)
0	MEAN	16.5	15.0	0.6	8.5	3.6
	STD	2.0	1.5	0.7	8.6	4.4
	STDERR	0.4	0.3	0.2	1.9	1.0
	N	20	20	20	20	20
2	MEAN	16.9	14.8	1.3*	11.4	8.2
	STD	2.5	1.9	0.9	13.0	5.9
	STDERR	0.6	0.4	0.2	3.0	1.3
	N	19	19	19	19	19
10	MEAN	16.3	14.1	1.3*	13.1	9.3*
	STD	2.4	2.5	0.9	15.6	7.1
	STDERR	0.6	0.7	0.2	4.0	1.8
	N	15	15	15	15	15
45	MEAN	16.8	14.3	0.7	14.6	5.5
	STD	2.1	2.8	0.9	17.7	7.0
	STDERR	0.5	0.6	0.2	4.1	1.6
	N	19	19	19	19	19

* : P <= .05, TWO TAILED DUNNETT T ON RAW DATA.
# : P <= .001, BARTLETT TEST FOR VARIABILITY.

Terminal and necroscopic evaluations: Testes weights and sperm (from epididymus) *in vitro* movement parameters were determined for 8 controls (all fertile matings), 2 LD (1 fertile and 1 infertile mating), 5 MD (4 infertile matings and 1 with no evidence of mating) and 1 HD (infertile mating); there were no obvious differences distinguishing the infertile matings (duloxetine-treated) from fertile (control) matings. However, sperm counts were not provided and testes and epididymus were not examined histopathologically. [NB As noted above, hypospermatogenesis was noted for 2/10 male rats (at each dose) treated for 6 mo with dietary doses equivalent to ~10 and ~50 mg/kg/d.]

2. ***Study title: A 10-week female basic fertility study of LY248686 [duloxetine] hydrochloride administered by oral gavage to female CD rats.***

Key study findings:

- Decreased maternal body weight, weight gain, and food consumption at HD (45 mg/kg);
- No effects on fertility parameters;
- Decreased live-born pups at HD;
- Dramatically decreased 1-day survival at HD;
- Decreased body weights of HD pups throughout 21-d post-partum period.

Study no.: R15590.
Volume #, and page #: EDR file Tox28.pdf; 127 pages.
Conducting laboratory and location: Lilly Research Labs, Greenfield, IN.
Date of study initiation: October 1, 1990, terminating December 9, 1990.
GLP compliance: yes, see page 3.
QA reports: yes, see page 2.
Drug, lot #, and % purity: duloxetine hydrochloride; lot no. 508NKO; — (+) enantiomer free base.
Formulation/vehicle: oral gavage of suspension in 10% aqueous acacia solution (with simethicone emulsion added to reduce foaming); 5 ml/kg.

Methods:

Species/strain: CD rats — :CD (SD), [ ]. ~9-wk old virgin females, weighing ~200 g, and adult males for breeding.
Doses employed: 0, 2, 10, 45 mg/kg/d of duloxetine (recalculated weekly).
Route of administration: oral gavage, 5 ml/kg.
Study design: males were untreated; females were treated for 10 weeks total, 2-wk pre-mating followed by up to 2-wk mating, 3-wk gestation, and 3-wk lactation; cohabitation was for 2 wk or until mating was confirmed by copulatory plugs and sperm in vaginal lavage; females that did not mate during first round were cohabited with a different proven male for an additional wk.
Number/sex/group: 20 females/group; at least 20 females/group.
Parameters and endpoints evaluated: survival and clinical signs daily, body weight, food consumption, and EFU weekly, mating performance and fertility, reproduction parameters, through lactation.

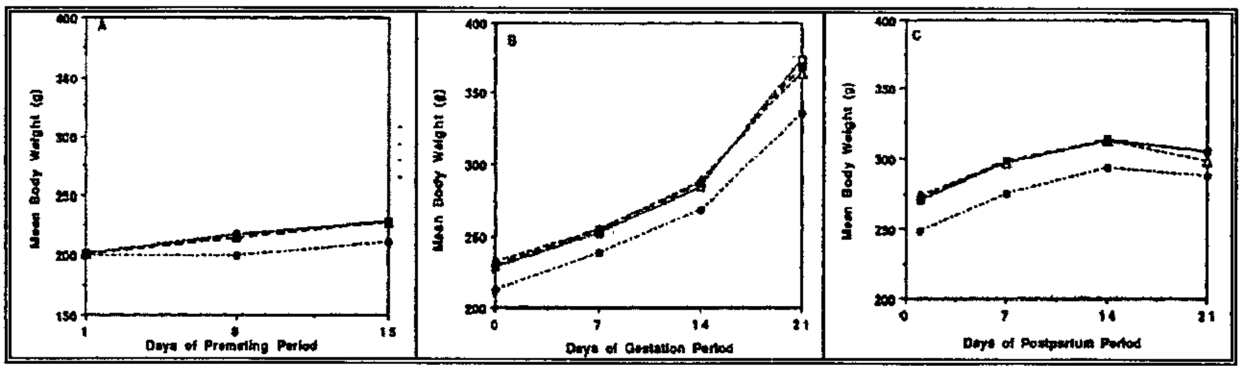
Results:

Mortality: none clearly directly related to duloxetine treatment. However, 2 HD females died during the study: 1 (#3055) died on pre-mating day 3, with histopathology suggesting "trauma-induced tracheobronchitis following accidental tracheal introduction during gavage;" the second (#3053) was killed in moribund condition on post-mating day 11, with histopathology suggesting "foreign body pneumonia secondary to aspiration, possibly following a gavaging accident."

Clinical signs: excessive salivation, 10-30 min after dosing, with dose-related frequency at MD and HD.

Body weight: At HD, body weight gains were decreased during weeks 1 and 2 of premating period (↓~50% compared with control over the 2-wk period) and HD females weighed ~7% less than controls at the start of mating. Although body weight gains in HD group paralleled those in control (and LD and MD) group during early gestation and post-partum, body weight gain from GD214-20 was decreased ~25% compared with controls and body weights of HD females remained lower than controls throughout the study (↓~7% at GD0 and post-partum days 1, 7, 14, and 12).

Figure 24. Duloxetine at 45 mg/kg/d, administered as the HCl salt by oral gavage, decreased body weight gain in female CD rats premating. The lowest curve in each panel represents the HD group. [Sponsor's graphs excerpted directly from this submission.]



Food consumption: Decreased food consumption mirrored decreased body weights at HD; ↓32% and ↓16% at pre-mating days 7 and 14; ↓10%, ↓10%, and ↓18% at GD 6, 13, and 20; but no different from control post-partum.

Toxicokinetics: not performed in this study.

Maternal reproductive parameters: There were no treatment-related effects on mating, precoital period or fertility, gestation length or litter size (see Table 32, below). 2 HD females (#3060 and #3066) had disrupted cycles (prolonged diestrus) after 1 wk of treatment; however, both mated in 2 days and delivered live pups.

Although litter sizes were not affected by duloxetine treatment, the live birth index (liveborn pups as % of litter size) was slightly lower for HD, 90%, compared with 99% for controls. Clinical observations common in HD litters included cold, thin, and pale or bluish in color. Survival for 24 hr after birth was dramatically decreased at HD, 62%, compared with 99% for controls. Survival of HD pups decreased another ~10% during the first week and day 21 survival was 52%, compared to 99% for controls.

Body weights of HD pups were ~20% lower than controls at day 1 and remained lower through day 21, when they were ~10% less than controls.

Table 32. Sponsor's tables of mating and fertility indices (left panel) and time to mating (precoital period), gestational length, and litter size (right panel) for female rats administered duloxetine as the HCl salt by oral gavage. [Excerpted directly from this submission.]

LY248686 DOSE (MG/KG/DAY)	STATISTIC	MATING INDEX(a)	FERTILITY INDEX(b)
0	COUNT	20	17
	NO. OBS	20	20
	PERCENT	100.00	85.00
2	COUNT	19	17
	NO. OBS	20	19
	PERCENT	95.00	89.47
10	COUNT	20	17
	NO. OBS	20	20
	PERCENT	100.00	85.00
45	COUNT	19	18
	NO. OBS	19	19
	PERCENT	100.00	94.74

* : P <= .05, TWO TAILED FREEMAN-TUKEY, BONFERRONI Z.
PER COMPARISON ERROR RATE = .0083333(VARIABLE).

LY248686 DOSE (MG/KG/DAY)	STATISTIC	PRECOITAL PERIOD (DAYS)	GESTATION LENGTH (DAYS)	LITTER SIZE(a)
0	MEAN	2.6	21.5	14.6
	STD	1.1#	0.6	1.2
	STDERR	0.2#	0.2	0.3
	N	20	17	17
2	MEAN	2.2	21.5	13.2
	STD	1.0#	0.5	3.7
	STDERR	0.2#	0.1	0.9
	N	19	17	17
10	MEAN	2.6	21.3	13.9
	STD	1.8#	0.5	1.9
	STDERR	0.3#	0.1	0.5
	N	20	16	16
45	MEAN	2.9	21.6	13.7
	STD	2.4#	0.6	2.2
	STDERR	0.6#	0.1	0.5
	N	19	17	17

* : P <= .05, TWO TAILED DUNNETT T ON RANKED DATA.
# : P <= .001, BARTLETT TEST FOR VARIABILITY.
NOTE: RAW DATA TABLED, RANKS TESTED.

Terminal and necroscopic evaluations: There were no overt malformations in offspring that survived or in those that died and were examined externally.

Summary of individual study findings: See Key Findings, above.

B. Embryo-fetal development (Segment II) studies in rats and rabbits.

1. ***Study title: A developmental toxicity study of LY248484 [duloxetine] hydrochloride administered orally to CD rats.***

Key study findings:

- Decreased maternal body weight, weight gain and food consumption at HD (45 mg/kg/d);
- Increased pre- and post implantation losses at HD;
- Decreased fetus weights at HD;
- No evidence of teratogenicity.

Study no.: R04490.
Volume #, and page #: EDR file Tox26.pdf; 369 pages.
Conducting laboratory and location: Lilly Research Laboratories, Greenfield, IN.
Date of study initiation: October 1, 1990, terminating on October 26, 1990.
GLP compliance: yes, see page 3.
QA reports: yes, see page 2.
Drug, lot #, and % purity: duloxetine hydrochloride; lot no. 508NKO; — pure.
Formulation/vehicle: oral gavage of suspension in 10% aqueous acacia solution (with simethicone emulsion added to reduce foaming); 10 ml/kg.

Methods:

<u>Species/strain:</u> virgin female CD rats — :CD (SD), [ ], ~9 wk old, 207 ± 9.5 g (mean ± SD) at start.
<u>Doses employed:</u> 0, 2, 10, 45 mg/kg/d (based upon GD6 body weights); based upon oral Segment II study (R02989) using duloxetine doses of 0, 3, 15, and 55 mg/kg/d, as maleate salt; Sponsor claims maternal toxicity (decreased weight gain and food consumption) at MD and HD, and decreased fetal weight at HD, but no effect on fetal viability or morphology at any dose tested. [K. Davis-Bruno's review of this study essentially confirmed the Sponsor's interpretation, specifically that there was no treatment-related teratogenicity.]
<u>Route of administration:</u> oral gavage.
<u>Study design:</u> females were mated with untreated adult male rats of same source and stock, then randomly (blocked by day of conception, when copulatory plug was expelled) assigned to dosing groups and housed individually, with food and water *ad libitum*; daily oral gavage administration on gestational days (GD) 6-17; females were killed (CO_2) on GD20.
<u>Number/sex/group:</u> 25 mated (copulatory plug expelled) females/group.
<u>Parameters and endpoints evaluated:</u> daily for survival and clinical signs of toxicity; eye exams pre-test and prior to termination at GD20; physical exams, body weight and food consumption on GD 0, 6, 10, 14, 18, and 20; net weight was terminal weight minus uterine weight; at termination on GD20, ovaries were examined for corpora lutea; uteruses were weighed and examined for implantations, live/dead fetuses, resorptions; live fetuses were weighed, examined externally for anatomical anomalies and gender, then ~half each litter was examined for visceral abnormalities, the other half for skeletal abnormalities.

Statistical analysis: by liter; p<0.05, 2-tail.

Results:

Maternal parameters:

Maternal mortality: no maternal deaths.

Maternal clinical signs: salivation in all rats at HD (45 mg/kg/d).

Maternal body weight and food consumption: decreased maternal body weight, weight gain and food consumption at HD (45 mg/kg/d). Although all groups weighed the same at GD 0 and 6 (before dosing was initiated), HD dams weighed significantly (~10%) less than controls at all subsequent days. Maternal food intake was also decreased at HD, ↓31% during GD6-9, ↓17% during GD10-13, ↓12% during GD14-17, but equal to controls at GD 18-19 (after last dosing on GD17).

Maternal reproduction parameters: increased pre-implantation loss at HD, with implantation sites for only 85% of the corpora lutea, compared with 94% in controls; increased post-implantation loss at HD due to increased early resorptions, reflected in ~9% total resorptions, compared with ~4% in controls.

Table 33. Maternal variables for Segment II study of 0, 2, 10, and 45 mg/kg oral (gavage) doses of duloxetine (as the hydrochloride) in rats. [Sponsor's values.]

PARAMETER	DOSE, mg/kg/d (gestational D6-17)			
	0	2	10	45
Total pregnancy rate	22/25	20/25	21/25	21/25
Mortalities	0	0	0	0
Pregnant Females at termination	22	20	21	21
Mean implantation sites	14.0	13.9	13.9	12.9
Mean corpora lutea	15.0	15.0	14.9	15.2
Pre-implantation loss, %, per dam	5.9	6.7	6.0	15.0*
Mean live fetuses	13.5	13.2	13.0	11.7
Mean dead fetuses	0.0	0.0	0.0	0.0
Mean early resorptions	0.5	0.7	0.9	1.1*
Mean late resorptions	0.0	0.0	0.0	0.1
Total resorptions, %, per litter	3.7%	4.9%	6.4%	8.7%*

Toxicokinetics: not performed in this study.

Fetal parameters:

Fetal weights: decreased slightly (4% for both males and females, but significant for females and combined sexes, only) at HD (45 mg/kg/d) compared with controls.

Fetal terminal and necroscopic evaluations: Malformations were rare, occurring in only 1, 2, 0, and 1 fetus (and litter) in controls, LD, MD, and HD, respectively. The control fetus was missing its jaw and mouth (external) and several related skeletal parts (mandible and incisor; maxilla and incisor). Two LD fetuses had malformations, one evidenced excess volume of amnion fluid (external) and the other (a runt) was missing thoracic vertebral arch and centrum. A single HD fetus had intestines protruding into umbilicus (external). Deviations and variations, especially skeletal, were more common, but did not show treatment-related increases.

Table 34. Embryo/fetal variables for Segment II study of 0, 2, 10, and 45 mg/kg oral (gavage) doses of duloxetine (as the hydrochloride) in rats. [Sponsor's values.]

PARAMETER	DOSE, mg/kg/d (gestational D6-17)			
	0	2	10	45
Mean live fetuses/litter	13.5	13.2	13.0	11.7
Males per litter	6.9	5.9	9.1	6.0
Females per litter	6.6	7.4	5.9	5.7
Mean % males	51.4%	44.2%	55.1%	53.2%
Mean fetus weights, g	3.51	3.64	3.52	3.36*
Total litters examined	22	20	21	21
Total fetuses examined for external abnormalities	296	264	272	248
Total fetuses examined for visceral abnormalities	155	137	142	127
Total fetuses examined for skeletal abnormalities	141	127	130	119
Total fetuses with malformations (litters)	1 (1)	2 (2)	0	1 (1)

2. ***Study title: A developmental toxicity study of LY248484 [duloxetine] hydrochloride administered orally to New Zealand white rabbits.***

Key study findings:

- Decreased maternal food consumption and body weight gains at HD (45 mg/kg/d);
- Slightly decreased early resporptions and post implantation loss at HD, with increased number of runts;
- Decreased fetus weights at HD;
- No evidence of teratogenicity.

Study no.: B09190.
Volume #, and page #: EDR file Tox27.pdf; 336 pages.
Conducting laboratory and location: Lilly Research Laboratories, Greenfield, IN.
Date of study initiation: November 12, terminating on December 14, 1990.
GLP compliance: yes, see page 9.
QA reports: yes, see page 8.
Drug, lot #, and % purity: duloxetine hydrochloride, lot no. 508NKO, — as HCl salt, — as the (+) enantiomer of the free base.

Formulation/vehicle: oral gavage of suspension in 10% aqueous acacia solution (with simethicone emulsion added to reduce foaming); 10 ml/kg.

Methods:

Species/strain: virgin female New Zealand white rabbits (Hra: (NZW), [ ]
~6.5 mo old and 3.40 ± 0.30 kg (mean ± SD) at start.
Doses employed: 0, 2, 10, and 45 mg/kg/d (GD6-18, based upon GD6 weights); based upon oral Segment II study (B00189) using duloxetine doses of 0, 3, 15, and 75 mg/kg/d, as maleate salt; Sponsor claims maternal toxicity (decreased weight gain and food consumption) at MD and HD, salivation at HD, and decreased fetal weight at HD, with secondary skeletal retardation of pubis, but no effect on fetal viability at any dose tested. [K. Davis-Bruno's review of this study essentially confirmed the Sponsor's interpretation, specifically that there was no treatment-related teratogenicity.]
Route of administration: oral gavage.
Study design: habituated females were randomly assigned to treatment groups, treated with chorionic gonadotropin (to induce ovulation), and mated with male breeders (same stock and source); viable sperm in vaginal lavage was designated GD0; rabbits were housed individually, with food and water *ad libitum*; mated females were dosed GD6-18, and were killed on GD28.
Number/sex/group: 20 mated females/dose.
Parameters and endpoints evaluated: daily for survival and clinical signs of toxicity; physical exams, body weight and food consumption on GD 0, 6, 13, 19, 24, and 28; net weight gain was weight minus uterine weight; at termination on GD28, carcasses were examined grossly; ovaries were examined for corpora lutea; uteruses were weighed and examined for implantations, live/dead fetuses, resorptions; live fetuses were weighed, examined externally for anatomical anomalies and gender, then ~half each litter was examined for visceral abnormalities, the other half for skeletal abnormalities.
Statistical analysis: by liter; $p<0.05$, 2-tail.

Results:

Maternal parameters:

Maternal mortality: The Sponsor found no compound-related deaths, however, several rabbits failed to complete the study:

- controls #35600 died on GD13 (pregnant; gavage error lesion); #35440, delivered early on GD28 (preceded by decreased body weight and food consumption); # 35460, removed from test on GD3 (no corpora lutea, no fetuses or resorptions; complete fracture of right tibia) before dosing began and not included in results.
- LD #35781 died on GD8 (pregnant; gavage error lesion),
- HD #35353 aborted and was killed on GD23 (gavage error lesion; preceded by decreased body weight and food consumption; no fetal anomalies detected on external exam).

Maternal clinical signs: none.

Maternal body weight and food consumption: decreased food consumption at HD (45 mg/kg/d), decreased maternal body weight, weight gain and food consumption at HD (45 mg/kg/d). Although HD dams ate slightly (~7%) more than controls during GD 0-5, they ate significantly less than controls during dosing, ↓15% during GD6-12 and ↓28% during GD13-18, but equal to controls at GD 19-27 (after last dosing on GD18). This decrease in food consumption was accompanied by decreased body weight gains, ↓72% during GD6-12 and ↓71% during GD13-18, but only 19% less than controls at GD 19-27 (after last dosing on GD18). However, these differences were not significant in the analysis of covariance (co-variate = GD0 body weight) performed by the Sponsor.

Maternal reproduction parameters: Duloxetine treatment did not adversely affect any maternal reproductive parameters, and tended to decrease early resorptions and post-implantation loss (see table, below).

Table 35. Maternal variables for Segment II study of 0, 2, 10, and 45 mg/kg oral (gavage) doses of duloxetine (as the hydrochloride salt) in rabbits. [Sponsor's values.]

PARAMETER	DOSE, mg/kg/d (gestational D6-18)			
	0	2	10	45
Total pregnancy rate	18/20	18/20	17/20	20/20
Drug-related mortalities	0	0	0	0
Gravid rabbits completing study	16	17	17	19
Mean implantation sites	7.3	7.5	7.8	7.7
Mean corpora lutea	11.9 [1]	11.1	12.2	11.5
Pre-implantation loss, %, per dam	32.1% [1]	27.2%	31.6%	29.2%
Mean live fetuses (%)	6.6 (86%)	7.1 (94%)	7.5 (93%)	7.4 (95%)
Mean dead fetuses	0.0	0.0	0.0	0.0
Mean early resorptions (%)	0.4 (12%)	0.4 (5.7%)	0.3 (6.1%)	0.1 (1.8%)
Mean late resorptions (%)	0.3 (2.6%)	0.1 (0.7%)	0.1 (0.6%)	0.3 (2.9%)
Total resorptions (post-implantation loss), per litter (%)	0.7 (14%)	0.5 (6.4%)	0.4 (6.7%)	0.4 (4.7%)

[1]: n=15; 1 female had regressed corpora lutea and was omitted from these calculations and subsequent fetus analysis.

Toxicokinetics: not done.

Fetal parameters:

Fetal weights: Although the tendency for decreased (~7%) fetal weights at the HD was not statistically significant, there was an increased incidence of runts at the HD, 10% of live fetuses, compared with 0 or 2% in other groups (see table, below). [ND There was a tendency for decreased early post-implantation loss, especially at HD, see above.]

Fetal terminal and necroscopic evaluations: Visceral and skeletal malformations were rare, with a tendency for higher incidence in drug-treated groups: 1) none in controls; 2) 2 LD fetuses from different litters had visceral, cardiovascular malformations, 1 a male with a fused aorta/pulmonary artery, the other a female missing a subclavian artery; 3) 5 MD fetuses from 2 litters had skeletal malformations, 4 fetuses (2 males, 2 females) in 1 litter had 1 or more of fused cervical vertebral arch, scrambled presacral vertebral centrum, fused small, missing or extra thoracic vertebral arch, and 1 male fetus in another litter had curvature of the vertebral column; 4) 3 HD fetuses from 3 litters had cardiovascular or skeletal malformations, 1 male runt was missing a subclavian artery, 1 female fetus was missing 2 "fingers," and another male fetus had displaced and fused occipital calvaria and exoccipital bones.

Table 36. Embryo/fetal variables for Segment II study of 0, 2, 10, and 45 mg/kg oral (gavage) doses of duloxetine (as the hydrochloride salt) in rabbits. [Sponsor's values.]

PARAMETER	DOSE, mg/kg/d (gestational D6-18)			
	0	**2**	**10**	**45**
Total litters with live fetuses	15	17	17	19
Mean live fetuses/litter	7.1	7.1	7.5	7.4
Males (%)	**3.3 (47%)**	**3.8 (57%)**	**3.8 (55%)**	**3.7 (51%)**
Females (%)	**3.7 (53%)**	**3.2 (43%)**	**3.6 (45%)**	**3.7 (49%)**
Mean fetus weights, g	**39.3**	**40.8**	**41.0**	**36.3**
Runts/litter (%)	**0.0 (0%)**	**0.2 (2%)**	**0.0 (0%)**	**0.8 (10%*)**
Total fetuses examined for external abn	116	121	128	153
Total fetuses examined for visceral abn	108	120	127	140
Total fetuses examined for skeletal abn	108	120	127	140
Malformation incidences:				
Visceral, fetuses (litters)	0	2 (2)	0	1
Skeletal, fetuses (litters)	0	0	5 (2)	2 (2)
Visceral or skeletal	0	2 (2)	5 (2)	3 (3)
Fetuses/litter with malformations (%)	0.0 (0.0%)	0.1 (1.8%)	0.3 (4.6%)	0.2 (1.6%)
Litters with malformations (%)	0 (0.0%)	2 (12%)	2 (12%)	3 (16%)
Fetuses/litter with deviations (%)	0.6 (9.3%)	0.2 (6.5%)	0.4 (4.0%)	0.8 (10%)
Litters with deviations (%)	6 (40%)	4 (24%)	5 (29%)	6 (32%)
Fetuses/litter with variations (%)	3.9 (54%)	5.2 (68%)	5.7 (75%)	4.7 (64%)
Litters with variations (%)	12 (80%)	16 (94%)	17 (100%)	18 (95%)

C. [Segment III] Study title: A fertility and developmental study of duloxetine hydrochloride (LY248686 hydrochloride) administered orally to female CD rats.

Key study findings:

- Decreased F0 maternal body weight, weight gain, and food consumption at HD (30 mg/kg);
- Increased F1 mortality: ↓ live-birth index, ↓24-hr survival, ↓1 week survival, ↓ pup weights from birth through post-natal day 14 at HD;
- Increased acoustic startle response at day 55 in HDF1 males;
- Decreased habituation of locomotor activity in HDF1 males at day 30 and HDF1 males and females at day 60;
- Tendency for poorer acquisition and retention of passive avoidance in HDF1 males at day 60;
- No effects on reproductive performance of F1 offspring.

Study no.: R25993 and R26193 (F1 generation study).
Volume #, and page #: EDR file Tox35.pdf; 518 pages.
Conducting laboratory and location: Lilly Research Labs, Greenfield, IN.
Date of study initiation: August 25, 1993, terminating February 24, 1994.
GLP compliance: yes, but study R26193 not QAed, see page 2.
QA reports: yes, but not study R26193, see page 2.
Drug, lot #, and % purity: duloxetine hydrochloride; lot no. CTM00027; — , total enantiomers free base.
Formulation/vehicle: oral gavage of suspension in 10% aqueous acacia solution (with simethicone emulsion added to reduce foaming); 10 ml/kg.

Methods:

Species/strain: CD rats (— CD (SD), C] ~9-wk old virgin females, weighing ~225 g, and adult males for breeding.
Doses employed: 0, 2, 10, 30 mg/kg/d of duloxetine (recalculated ~weekly, except not for GD21).
Route of administration: oral gavage, 5 ml/kg.
Study design: F0 males were untreated; F0 females were treated for ~10 weeks total, 2-wk pre-mating followed by up to 2-wk mating, 3-wk gestation, and 3-wk lactation; cohabitation was for 2 wk or until mating was confirmed by copulatory plugs and sperm in vaginal lavage. At post-partum day 1, litters were randomly culled to 8 pups/litter and 1 pup/sex/litter was randomly chosen for F1 behavioral testing between days 6 and 70; and a reproduction trial was conducted. F1 dams delivered and maintained F2 offspring through post-partum day 1, when F1 rats were necropsied and reproductive organs were examined for histopathology.
Number/sex/group: 20 females/group; at least 20 females/group.
Parameters and endpoints evaluated: survival and clinical signs daily, body weight, food consumption, and EFU weekly, mating performance and fertility, reproduction parameters, through lactation; F1: behavioral testing and reproductive performance.

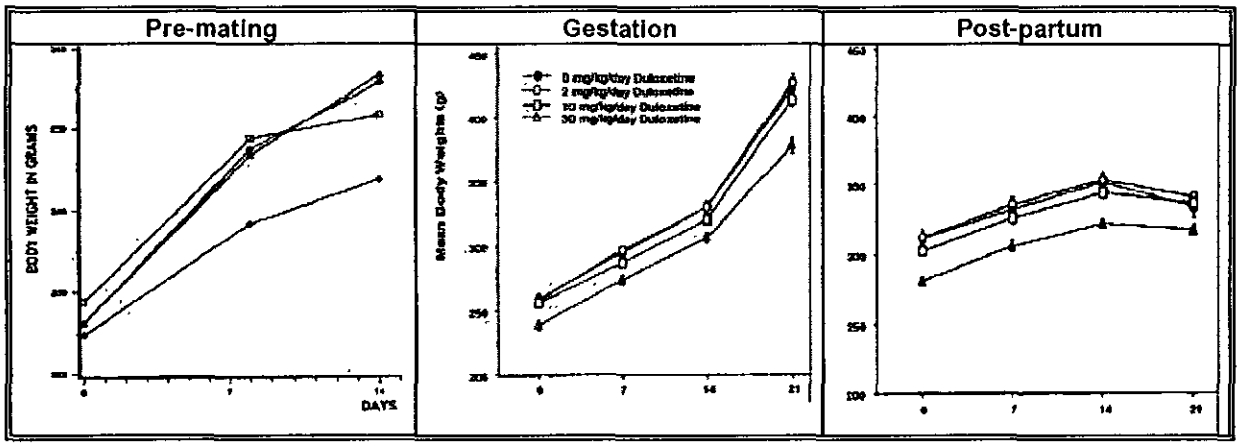
Results:

F0 mortality: none; all F0 female survived to scheduled termination.

F0 clinical signs: none that were treatment-related.

F0 body weight: At HD, body weight gains were decreased during weeks 1 and 2 of premating period (↓35% compared with control over the 2-wk period) and HD females weighed ~5% less than controls at the start of mating and ~8% less than controls on the first day of gestation. Although body weight gains in HD group paralleled those in control (and LD and MD) group during early gestation and post-partum, body weight gain from GD14-21 was decreased ~25% compared with controls and body weights of HD females remained lower than controls throughout the study (↓~8% at GD0 and ↓10%, ↓8, ↓8, and ↓5% on post-partum days 1, 7, 14, and 21, respectively).

Figure 25. Duloxetine at 30 mg/kg/d, administered as the HCl salt by oral gavage, decreased body weight gain in female CD rats premating. The lowest curve in each panel represents the HD (45 mg/gk/d) group. [Sponsor's graphs excerpted directly from this submission.]



F0 Food consumption: Decreased food consumption mirrored decreased body weights at HD; ↓6%, ↓11%, and ↓14% at GD 7, 14, and 21; and ↓11% and ↓14% compared with controls at 7 and 14 days post-partum, respectively.

Toxicokinetics: not performed in this study.

F0 maternal reproductive parameters: There were no treatment-related effects on mating, precoital period or fertility, gestation length or litter size.

F1 mortality, clinical signs, and body weights: Although litter sizes were not significantly affected by duloxetine treatment, the live-birth index (live-born pups, as % of litter size) was slightly lower for HD, 93%, compared with 99% for controls. **Clinical observations common in HD litters included cold,**

small, dead, stomach with little of no milk, and pale or bluish in color. Survival for 24 hr after birth was dramatically decreased at HD, 78%, compared with 100% for controls. Survival of HD pups decreased another ~20% during the first week post-partum. Body weights of HD pups were 14% lower than controls at day 1 and remained lower through day 14, when they were ~10% less than controls; by day 21 HD pup weights were not different from controls.

After weaning, 1 F1 male and 1 F1 female from litters (18, 16, 17, and 14 F0 litters from control, LD, MD and HD groups, respectively) were allowed to develop and were evaluated for performance in several behavioral tasks and for reproductive performance. Body weights for these F1 offspring of drug-treated dams were not different from controls throughout the rest of the study, at day 105, when mating was initiated, or during gestation.

F1 developmental morphological markers: no effect on incisor eruption or eye opening. Incisor eruption was monitored on post-natal days 11, 12, and 13; there was no treatment-related effect on latency and incisors had erupted in 80-90% of pups in each group by day 13. Eye opening was monitored on post-natal days 15, 16, and 17; there was no treatment-related effect on latency and eyes had opened in ~90% of pups in each group by day 17.

F1 behavioral tests:

- Negative geotactic responding (time [up to 60 sec] to turn from head down to head up position, on post-natal days 6, 7, and 8). **HDF1 males performed poorer** than other groups **on day 7**; the fraction of male pups responding approximately doubled from days 6 to 7 in the other groups (and latencies decreased), but the fraction of HDF1 males responding increased only slightly (↑30%) and latencies were essentially unchanged. By day 8, HDF1 males were performing similarly to controls (86%, compared with 94% of controls). Female pups showed an increased incidence of responding across the test days regardless of treatment, with ~80% of pups responding by day 8.

- Auditory startle habituation (5 consecutive blocks of 10 trials each, on post-natal days 19 and 55): On post-natal day 19, there were no treatment-related differences in initial peak response or latency to peak response, and peak response decreased similarly across the 5 blocks for all groups. **On post-natal day 55** the initial peak response was considerably larger for all groups and peak response decreased similarly across the 5 blocks for all groups. However, **overall peak response was ~40% higher (and latency was lower) for HDF1 males.** [NB The Sponsor also notes increased peak startle response in MDF1 females on day 55 in block 3 only, and in MDF1 males on day 19 in block 1 only, however, I do not find this compelling.]

- Locomotor activity in a figure-8 maze (4 consecutive 15 min blocks per day, days 30 and 60): **On day 30**, there were no treatment-related differences in initial activity and activity decreased similarly during the 4 test blocks; **HDF1 males were slightly (~50%) more active than controls during block 4 only. On day 60**, there were no treatment-related differences in initial activity and activity decreased similarly during the 4 test blocks; **HDF1 males and females were slightly (~30%, not significantly) more active than controls at block 4**. These increases in activity, relative to controls, toward the ends of the trials may reflect decreased habituation to the novelty of the environment, rather than simply increased activity.

- Passive avoidance (3 consecutive 3-min training trials [separated by 1-min intervals] on day ~60, 1 retention trial on following day): There were no treatment-related differences in initial latency to cross and latency increased similarly during the 3 test blocks. HDF1 males seemed to acquire the task slightly more slowly (based upon non-significantly ~20% shorter latency than controls at trial 2) and retention was slightly poorer (based upon ~30% decreased latency and ~2-fold increased number of crossings, but neither significant). HDF1 females also seemed to acquire the task slightly more slowly, but retention was relatively poor for all female groups. These slight (non-significant) increases in response rate may reflect the decreased habituation seen for locomotor activity.

F1 fertility and reproduction parameters: no effect on mating or fertility indices, time to mating, gestation length, litter size, or post-natal day 1 pup weights. No evidence of gross malformations in F2 progeny that were born dead or died during the lactation period.

F1 pathology: No treatment-related gross or histopathology findings (whole animal, ovary, uterus, vagina, testes, prostate, epididymis, or seminal vesicle) for F1 rats killed at 136 days of age.

D. Reproductive and developmental toxicology summary:
Duloxetine HCl was administered orally (by gavage) at doses of 0, 2, 10, and 45 mg/kg/day to male or female rats in separate studies to determine effects on mating and fertility. Although the HD decreased food consumption, body weight gain, and body weight, none of the doses (except MD in males) affected mating or fertility parameters. It should be noted that the MD (10 mg/kg) administered to males did decrease their fertility index and females that mated with them had increased early resorptions; however, there was no effect on HD males or females that mated with them, so the reliability of this observation is suspect.

Duloxetine HCl was administered orally (by gavage) at doses of 0, 2, 10, and 45 mg/kg/day to pregnant rats and rabbits throughout organogenesis to determine effects on embryo-fetal development. The HD decreased food consumption and body weight gain in both rats and rabbits. Effects on fetuses were limited to the HD: 1) increased pre- and post-implantation losses in rats; 2) slightly decreased early resorptions (and post-implantation loss) and increased number of runts in rabbits; 3) decreased fetus weights at HD in both rats and rabbits; and 4) no evidence of teratogenicity in either rats or rabbits.

Duloxetine HCl was administered orally (by gavage) at doses of 0, 2, 10, and 30 mg/kg/day to pregnant rats during gestation, through delivery and lactation, to determine effects on the F1 progeny. The HD decreased F0 maternal body weight, weight gain, and food consumption. Effects on F1 offspring were confined to those from F0 dams that had been treated with the HD: 1) increased F1 mortality, including decreased live-birth index, decreased 24-hr survival, decreased survival to 1 week; 2) decreased pup weights from birth through post-natal day 14; 3) behavioral responses consistent with what the Sponsor refers to as increased reactivity, including increased acoustic startle peak response at post-natal day 55 in HDF1 males, decreased habituation of locomotor activity in HDF1 males at post-natal days 30 and 60 and in HDF1 females at day 60 only, and a tendency for poorer acquisition and retention of passive avoidance in HDF1 males at day 60; but 4) no effects on F1 reproductive performance.

In the fertility study of female rats, dosing of dams with 0, 2, 10, and 45 mg/kg/day was continued through lactation. Effects on offspring were confined to the HD: 1) decreased live-born pups; 2) dramatically decreased 24-hr survival; and 3) decreased pup weights throughout the 21-d post-partum (lactation) period.

E. Reproductive and developmental toxicology conclusions:
Duloxetine did not alter mating or fertility when administered to either male or female rats.

Duloxetine was not teratogenic in rats or rabbits, however, it did increase post-implantation loss in rats and decrease fetal weights in both rats and rabbits at 45 mg/kg/day. A dose of 30 mg/kg/d appeared to make F1 offspring, especially males, more reactive, as demonstrated by increased acoustic startle response, decreased habituation of locomotor activity, and possibly decreased rate of acquisition and retention of a passive avoidance behavior.

F. Reproductive and developmental labeling recommendations:

Impairment of Fertility

Duloxetine administered orally to either male or female rats prior to and throughout mating at daily doses up to 45 mg/kg — .imes the maximum recommended human dose [MRHD] on a mg/m^2 basis) did not alter mating or fertility.

Pregnancy
Pregnancy Category C

In animal reproduction studies, duloxetine has been shown to have adverse effects on embryo/fetal and postnatal development.

When duloxetine was administered orally to pregnant rats and rabbits during the period of organogenesis, there was no evidence of teratogenicity at doses up to 45 mg/kg/day (— times the maximum recommended human dose [MRHD] on a mg/m^2 basis, in rats and rabbits, respectively). However, fetal weights were decreased at this dose, with a no-effect level of 10 mg/kg (— times the MRHD on a mg/m^2 basis, in rats and rabbits, respectively).

When duloxetine was administered orally to pregnant rats throughout gestation and lactation, the survival of pups to 1 day postpartum and pup body weights at birth and during the lactation period were decreased following maternal exposure to 30 mg/kg/day ('imes the MRHD on a mg/m^2 basis), with a no-effect level of 10 mg/kg. Furthermore, behaviors consistent with increased reactivity, such as increased startle response to noise and decreased habituation of locomotor activity, were observed in pups following maternal exposure to 30 mg/kg/day. Post-weaning growth and reproductive performance of the progeny were not affected adversely by maternal duloxetine treatment.

There are no adequate and well-controlled studies in pregnant women, therefore duloxetine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Labor and Delivery

[ ]

Nursing Mothers

[ ]

[ ]

[ ]

Pediatric Use

[ ]

APPEARS THIS WAY
ON ORIGINAL

VIII. SPECIAL TOXICOLOGY STUDIES:

A. Antigenicity of duloxetine was tested in Guinea pigs using an active systemic anaphylaxis test (ASA), a passive cutaneous anaphylaxis test (PCA), and an enzyme-linked immunosorbent assay (ELISA) (Tox40.pdf, Sept, 1996). 1. Duloxetine was not immunogenic, by multiple oral or subcutaneous administrations, with or without Freund's complete adjuvant; 2) the hypersensitivity-eliciting antigenicity of duloxetine was limited to ASA when immunized with a hapten-protein conjugate with adjuvant and then only by oral, not iv, challenge; not PCA in passively sensitized Guinea pigs, regardless of route.

B. Antigenicity of duloxetine was tested in mice using a passive cutaneous anaphylaxis test (PCA), and an enzyme-linked immunosorbent assay (ELISA) (Tox40.pdf, Sept, 1996). 1. Duloxetine was not immunogenic, by multiple oral or intraperitoneal administrations, with or without Freund's complete adjuvant; and no serum titers were detected by ELISA; 2) duloxetine did not possess hypersensitivity-eliciting antigenicity in mice, based upon lack of PCA in rats passively sensitized with the immunized mouse sera.

C. Summary and Conclusions: Duloxetine seems unlikely to pose an antigenicity risk.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

A. Overall Summary:

Pharmacodynamics: *In vitro*, duloxetine potently inhibits the reuptake of serotonin and norepinephrine, and has ~100-fold lower affinity for dopamine reuptake. Although some metabolites of duloxetine have significant affinity for the reuptake transporters, the major metabolites circulating in human plasma, 4-OH-duloxetine-glucuronide and 5-OH, 6-MeO-duloxetine sulfate, do not (Ki's >3000 nM). Consequently, these 2 major metabolites would not be expected to contribute to the pharmacological effect(s) of duloxetine in humans, however, the possiblity of toxic effects can not be eliminated. *In vivo*, duloxetine blocks serotonin and norepinephrine depletion by reuptake transporter substrate releasers, such as p-chloroamphetamine and 6-OH-dopamine, consistent with reuptake transporter inhibition by duloxetine.

The mechanism of action of duloxetine as an anitdepressant is unknown. As for other currently approved antidepressants, the actual mechanism of action is probably through (as yet unidentified/unexplained) compensatory mechanisms initiated by the direct effect(s) of the drugs. For duloxetine, the initiating effect is assumed to be blockade of reuptake of serotonin and norepinephrine, which results in enhanced serotonergic and noradrenergic activities in the CNS.

Duloxetine has been determined to have low affinity (Ki > 1000 nM) for other neuronal receptors and bindings sites *in vitro*. Consequently, side-effects mediated through other receptors would not be anticipated. Paul Andreason, the Medical Officer for this submission, noted hypertension as a potential risk. Although the binding affinities wouldn't necessarily predict cardiovascular side effects, the potentiation of norepinephrine's effects by duloxetine (by blockade of reuptake) is consistent with this side effect.

Safety Pharmacology: Duloxetine is a centrally, as well as peripherally, acting drug and was active in animal models that are predictive of antidepressant and antinociceptive (chronic pain) activities in humans. Although convulsions were seen at high doses in acute toxicity studies, duloxetine did not appear to be pro-convulsant; it did not potentiate pentylenetetrazol-induced seizures and appeared to decrease sensitivity to electrogenic seizures in mice.

Although potential for cardiac toxicity can not be ruled out completely, there is no pre-clinical evidence that would indicate a problem.

Limited testing in rats and monkeys did not indicate abuse liability: duloxetine was not self-administered by monkey trained to self-administer barbiturate; did not block barbiturate withdrawal signs; and did not produce withdrawal signs after discontinuation of repeated (4-week) dosing.

While not strictly a safety issue, duloxetine decreased food consumption in animal species (e.g., rats, mice, dogs).

Pharmacokinetics: Duloxetine (as the maleate salt) was rapidly (Tmax of 1-1.5 hr) and essentially completely absorbed (82% and 100%) following oral administration to rats and dogs. Especially at this low dose (5 mg/kg) duloxetine was extensively metabolized, with unchanged duloxetine accounting for only 7% and 1% of total systemic exposure for 24 hr after administration to rats and dogs, respectively.

At low doses (5-10 mg/kg) in rats and dogs, duloxetine (total radioactivity) was excreted ~25-30% in urine and ~60-70% in feces, after both oral and intravenous dosing. In monkeys, a 5 mg/kg oral dose was excreted ~60% in urine and ~30% in feces. In humans, a 20 mg/kg oral dose was excreted 70-80% in urine and 15-20% in feces.

Duloxetine is extensively metabolized in all animal species that were studied, including humans. The Sponsor has identified the glucuronide conjugate of 4-OH-duloxetine and the sulfate conjugate of 5-OH, 6-MeO-duloxetine as the major circulating metabolites in humans. This designation appears to be based upon the fraction of total radioactivity peak area (not the fraction of total radioactivity [ ]) from a pooled 10-hr plasma sample in study SAAZ. In that study, duloxetine accounted for 4%, 4-OH-duloxetine-glucuronide for 47%, and 5-OH, 6-MeO-duloxetine-sulfate for 22%; 2 other metabolites, dihydroxy/catechol-duloxetine-glucuronide and 6-OH, 5-MeO-duloxetine-glucuronide, accounted for 14 and 13%, respectively.

In order to get a better idea of how much of the systemic exposure in humans is accounted for by (or due to) these metabolites, I combined results from 3 human studies and estimated that: 1) unchanged duloxetine accounts for only 3% of the total systemic (plasma AUC) exposure to drug; 2) 4-OH-duloxetine-glucuronide accounted for another 27%; and 3) 5-OH, 6-MeO-duloxetine-sulfate accounted for another 12 %. These 3 molecular species probably account for ~40% of the systemic exposure to orally administered duloxetine HCl (at least at doses up to 60 mg).

Duloxetine was extensively metabolized in the animal species used for toxicological evaluation and the major (most prevalent) metabolites circulating in humans were not well covered by in the animals.

- 4-OH-duloxetine-sulfate conjugate (the circulating form in humans) was also detectable in the plasma of rats and mice, but not dogs. The amount of this metabolite was estimated in the plasma of rats and mice from 3-mo dietary studies (at doses comparable to those used in other toxicology studies). There appeared to be a considerable safety margin in mice for a 60-mg human dose at steady-state, but no safety margin in rats (<0.1).

- 5-OH, 6-MeO-duloxetine, which circulated as the sulfate conjugate in humans, was not detected in any form in the plasma of dogs, rats or mice. However, there was evidence that this metabolite was synthesized in all 3 animal species: a) the glucuronide conjugate was present in urine of rats and mice and the free metabolite was present in mouse urine; b) the metabolite was detected in feces of dogs, as free metabolite, and bile of rats, as the glucuronide.

General Toxicology: Repeated-dose toxicology studies of adequate duration and at MTDs were performed in rats and dogs. These studies showed no life-threatening toxicities at doses that decreased body weights.

In the 6-month dietary study in rats (dietary doses of 0.005, 0.02, or 0.08%), doses were limited by decreases in body weights (and food consumption) at HD (~50 mg/kg). The liver appeared to be the only target of toxicity, with slightly increased serum liver enzyme levels, increased liver weights, increased incidence and severity of midzonal vacuolation, and induction of some P450 isoforms. [Liver toxicity was also noted in the 2-yr carcinogenicity study: 1) increased occurrence of cholesterol granuloma; and 2) dose-related increase in multi-nucleated (3-40) hepatocytes (MNHs).]

In the 1-year study in dogs (3, 10, and 30 mg/kg/day orally, in a gelatin capsule), emesis and decreased food consumption at the HD limited doses. Aside from clinical signs (e.g., mydriasis, and slow or incomplete pupillary response), the drug-related findings were in the liver and were limited to slightly increased amount of secondary lysozomes and slight induction of CYP 2B at HD.

Genotoxicity: Duloxetine was adequately tested and negative in 2 out of 3 parts of the standard battery: in the Ames test for bacterial mutagenicity and the *in vivo* mouse micronucleus assay for clastogenicity. Two *in vitro* tests for clastogencity, chromosomal aberrations in CHO cells and "large" colony formation in L5178Y mouse lymphoma cells, were negative with and without metabolic activation when tested for a short duration (4 hr) of duloxetine exposure, but neither was followed up with a longer (~24 hr) treatment without activation, as recommended according to current guidelines. Additionally, each study was further flawed: in the CHO cell assay, only 100 (not 200) metaphases were counted per treatment and in the mouse lymphoma assay, large and small colonies were not reported separately, although the lack of effect on total seems unlikely to mask an increase in large colonies.

Additionally, duloxetine was not genotoxic in 2 other assays: unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes and *in vivo* sister chromatid exchange in Chinese hamster bone marrow.

Carcinogenicity: Although the carcinogenicity studies were performed prior to the establishment of the CAC within the Agency, the dietary doses used in the studies were based upon decreases in body weights or weight gains from dietary studies of at least 13-week duration and are in agreement with the currently accepted standards for dose-selection. Additionally, increased mortality was observed in male mice at the HD. Although decreases in food consumption accompanied the dose-limiting decreased body weights in rats, palatability did not appear to be an issue. It seems likely that decreased food consumption and decreased body weight would limit doses (at least in rats) regardless of route (see Safety Pharmacology).

This Reviewer agreed with the Sponsor that there were no duloxetine-related tumors evident in rats or male mice up to maximum tolerated doses (MTD); and that the positive carcinogenicity findings were hepatocellular adenomas and carcinomas in livers of high-dose females (~140 mg/kg). Concurrence was given by the Executive-CAC on July 30, 2002 (meeting minutes appended to this review).

Reproductive Toxicity: In separate Segment I studies, duloxetine was administered orally (by gavage) at doses of 0, 2, 10, and 45 mg/kg/day to male or female rats to determine effects on mating and fertility. Although the HD decreased food consumption, body weight gain, and body weight, none of the doses (except MD in males) affected mating or fertility parameters. It should be noted that the MD (10 mg/kg) administered to males did decrease their fertility index and females that mated with these MD maels had increased early resorptions; however, there was no effect on HD males or females that mated with them, so the reliability of this observation at MD is suspect.

In Segment II studies, duloxetine was administered orally (by gavage) at doses of 0, 2, 10, and 45 mg/kg/day to pregnant rats and rabbits throughout organogenesis to determine effects on embryo-fetal development. The HD decreased food consumption and body weight gain in both rats and rabbits. Effects on fetuses were limited to the HD: 1) increased pre- and post-implantation losses in rats; 2) slightly decreased early resorptions (and post-implantation loss) and increased number of runts in rabbits; 3) decreased fetus weights at HD in both rats and rabbits; and 4) no evidence of teratogenicity in either rats or rabbits.

In a Segment III study, duloxetine HCl was administered orally (by gavage) at doses of 0, 2, 10, and 30 mg/kg/day to pregnant rats during gestation, through delivery and lactation, to determine effects on the F1 progeny. The HD decreased F0 maternal body weight, weight gain, and food consumption. Effects on F1 offspring were confined to those from F0 dams that had been treated with the HD: 1) increased F1 mortality, including decreased live-birth index, decreased 24-hr survival, decreased survival to 1 week; 2) decreased pup weights from birth through post-natal day 14; 3) behavioral responses consistent with what the Sponsor refers to as increased reactivity, including increased acoustic startle peak response at post-natal day 55 in HDF1 males, decreased habituation of locomotor activity in HDF1 males at post-natal days 30 and 60 and in HDF1 females at day 60 only, and a tendency for poorer acquisition and retention of passive avoidance in HDF1 males at day 60; but 4) no effects on F1 reproductive performance.

Results from the Segment I fertility study of female rats (see above), where dosing was continued through lactation, confirm the Segment III study results. Effects on offspring were confined to the HD (45 mg/kg): 1) decreased live-born pups; 2) dramatically decreased 24-hr survival; and 3) decreased pup weights throughout the 21-d post-partum (lactation) period.

In conclusion, duloxetine did not alter mating or fertility when administered to either male or female rats. Duloxetine was not teratogenic in rats or rabbits, however, it did increase post-implantation loss in rats and decrease fetal weights in both rats and rabbits at 45 mg/kg/day. A dose of 30 mg/kg/d appeared to make F1 offspring, especially males, more reactive, as demonstrated by increased acoustic startle response, decreased habituation of locomotor activity, and possibly decreased rate of acquisition and retention of a passive avoidance behavior.

B. Pharmacology/Toxicology Issues and Recommendations:

1) Systemic exposures to major circulating human metabolite(s) were not covered in non-clinical toxicology species/studies:
   - 4-OH-duloxetine glucuronide represents approximately 27% of total systemic exposure to duloxetine and metabolites in humans, which is ~9-times the exposure to unchanged duloxetine (3% of total). There is ~30-fold safety margin in mice (e.g., NOEL ~50 mg/kg in the 2-yr carcinogenicity study), but less than 0.1-fold safety margin in rats (viz., in the 2-yr carcinogenicity study). 4-OH-duloxetine glucuronide was undetectable in dog plasma (6-mo and 1-yr studies), but was detected in dog urine and the unconjugated form was detected in dog feces.
   - 5-OH, 6-MeO-duloxetine sulfate represents 12% of total systemic exposure to duloxetine and metabolites in humans, which is 4-times the exposure to unchanged duloxetine. This metabolite (free or conjugated) was not detected in plasma of rats, mice or dogs. It was detected in urine of mice (as glucuronide and unconjugated) and rats (as glucuronide), and in feces of rats (as glucuronide) and dogs (unconjugated).
   - 2 other metabolites, 6-OH, 5-MeO-duloxetine glucuronide and diOH-duloxetine glucuronide, were only quantified at a single time point (10 hr after dosing) in humans, where their levels were each ~3-times that of unchanged duloxetine. These metabolites were not detected in plasma of rats, mice, or dogs; however, their inadequate quantification in human plasma does not clearly point to a need for coverage in pre-clinical studies.

➢ **I recommend** that both 4-OH-duloxetine glucuronide conjugate (which represented ~30% of total human systemic exposure and at most 0.1-fold coverage in rats) and 5-OH, 6-MeO-duloxetine sulfate (12% of human exposure, but undetected in plasma of any toxicology species) be qualified according to ICH Q3A Guideline on Impuritis (1996). Qualification should include: 1) *in vitro* genotoxicity testing, specifically the Ames test and *in vitro* chromosomal aberration test, and 2) a Segment II reproductive toxicity study, because the patient population will include women of child-bearing potential. It should be noted that intravenous, rather than oral, dosing would probably be necessary to achieve adequate plasma concentrations of these molecules.

2) Specifications set for 'mpurities in the clinical drug substance will require qualification: the [ ]
   - The — was only quantifiable in the carcinogenicity studies — and the Segment III reproductive toxicology study — [see table below]. I could not find any evidence that — occurred in humans or animals. The Sponsor argued in the Biopharm Summary of this submission that — *in vivo* was highly unlikely. Furthermore, the results of stability testing suggest that — does not occur *in vitro*. The Sponsor states that the [ ] is controlled in [ ] product).
   - The Sponsor says that [ ] has been decreased — in recent batches (since 1994) and was qualified through initial toxicology and subsequent clinical studies. However, this impurity was not

quantified in the analytical characterization reports included in the individual study reports (see Table 37, below).

Table 37. Table showing the amounts of impurities [ ] in toxicology batches of drug substance [data extracted from individual study analytical characterization reports].

Type (year)	Study #	Lot #
spec		
Ames test (1990)	Tox24	619NKO
In vitro chr ab (1990)	Tox30	619NKO
Ms micro (1990)	Tox20	619NKO
1-yr dog (1990)	Tox33	619NKO
		521NKO
6-mo rat (1990)	Tox31	508NKO
6-mo dog (1990)	Tox32	508NKO
Seg I (M) (1990)	Tox25	508NKO
Seg I (F) (1990)	Tox28	508NKO
Seg II (rat) (1990)	Tox26	508NKO
Seg II (rb) (1990)	Tox27	508NKO
Seg III (1993)	Tox35	CTM00027
Ms carc (1991)	Tox43	DPD13975
Rat carc (1991)	Tox44	DPD13975

ns: not specified.
nd: not detectable.
ns*: not specified, but total related substances were estimated at
ns**: not specified, but total related substances were estimated at
ns***: not specified, but total related substances were estimated at
1992.
ns****: not specified, but total related substances were estimated at

J

- **I recommend** that either: 1) data be submitted showing that the amounts of these impurities in the lots of drug substance used in pivotal toxicology studies (e.g., *in vitro* genotoxicity tests (Ames and chromosomal aberrations) and a segment II reproductive toxicity study) qualify these ~ impurities; or 2) specifications for these —mpurities be lowered to . — % so that qualification is not an issue; or 3) these —mpurities be qualified.

3) Although the amounts of —— present in the toxicology batches do not support the proposed specifications (see Table 37, above), qualification of these — will not be required, because the specifications were set at the limits proposed by ICH Guidance on Residual Solvents: QC3 (1997). Limits for —— , were set at — ppm specified in the Guidance). Limits for —— , were set at —— in accord with the Guidance.

4) The excipient hydroxypropyl methylcellulose acetate succinate (HPMCAS) was used in the to be marketed drug product; its pH-dependent solubility allowed duloxetine to pass through the acidity of the stomach (avoiding the formation of toxic acid-rearrangement products) and be absorbed from the intestine. Underivatized HPMC is very stable and is in common use in drug formulations, including oral formulations. The HPMCAS form, with common salts acetate and succinate attached through ester bonds, has recently been approved for use in a weekly oral dosing formulation of fluoxetine (Prozac DuraPac, NDA 21-235). The Sponsor for that NDA (Eli Lilly, again) declined to determine whether HPMCAS was absorbed in humans, for ethical reasons, but preclinical studies submitted in support of that NDA demonstrated that little or no HPMCAS (^{14}C-labeled on the succinate moiety) was absorbed from the gastro-intestinal tract of rats after a single oral dose of 1000 mg/kg (see P/T review by Barry Rosloff). For Prozac DuraPac, the weekly human intake of HPMCAS is —— the daily intake of HPMCAS for the MRHD (120 mg) of the current formulation of duloxetine is — regardless of the capsule strength used. Thus, the daily dose of HPMCAS is approximately 10-times greater for the duloxetine formulation currently under review, but the daily dose of — ng/kg/day is still quite low.

5) The *in vitro* chromosomal aberration test, part of the standard test battery according to the current ICH Guidance for Industry, S2B Genotoxicity, 1997, was inadequate. In the submitted study, duloxetine was negative for 4-hr treatment, with and without metabolic activation. However, only 100 (not 200) metaphases were counted per treatment. Furthermore, the study was not adequate, because this negative finding (without activation) should have been verified with a study using continuous treatment with duloxetine (without activation) for ~24 hr (1.5 cell doubling times), in accordance with the current ICH Guidance.

➢ **I recommend** that the negative results in this study be followed up with another study using a longer (~24-hr) treatment with duloxetine (without metabolic activation).

C. Recommendations for labeling

Below find the Sponsor's proposed labeling (A) followed by my recommendations for revised labeling (B) for each section.

4 pages redacted from this section of the approval package consisted of draft labeling

Pediatric Use

DRUG ABUSE AND DEPENDENCE

Physical and Psychological dependence

A. The Sponsor's proposed labeling:

B. My revised labeling recommendations:

X. APPENDIX/ATTACHMENTS:

E-CAC meeting minutes:

Date of Meeting: July 30, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Jim Farrelly, Ph.D., HFD-530, Alternate Member
Barry Rosloff, Ph.D., HFD-120, Team Leader
Linda H. Fossom, Ph.D., HFD-120, Presenting Reviewer

Author of Draft: Linda H. Fossom

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

NDA 21-427.
Drug Name: duloxetine hydrochloride.
Sponsor: Eli Lilly and Company.

Background: Duloxetine is an inhibitor of reuptake pumps for both norepinephrine and serotonin. It was not mutagenic/genotoxic in the standard battery; however, the negative results in the *in vitro* test for chromosomal aberrations after 4-hr treatment should have been verified with ~24-hr treatment, according to the ICH Guidance for Industry: S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. Additionally, 3 other tests did not indicate mutagenic/genotoxic potential.

Mouse Carcinogenicity Study: CD-1 mice were treated for 2 years with duloxetine hydrochloride in their diet at duloxetine concentrations of 0, 0.005, 0.01, 0.03, and 0.08%. The high-dose (HD) was adequate as a maximally tolerated dose (MTD), based upon decreased body weight gain (10%) in the 3-mo dose-ranging study and upon decreased body weights in both males (7%) and females (9%) and slightly (but significantly) increased mortality in males in this study.

Increased incidence of tumors was limited to benign endometrial stromal tumors in the uteruses and hepatocellular adenomas and carcinomas in the livers of HD females, only.

Rat Carcinogenicity Study: Fischer 344 rats were treated for 2 years with duloxetine hydrochloride in their diet at duloxetine concentrations of 0, 0.01%, 0.02%, and 0.05% (females only) or 0.08% (males only). The average daily doses were 0, 4.37, 8.50, and 35.8 mg/kg for males and 0, 5.43, 10.6, and 26.7 mg/kg for females. The HDs appeared to be adequate as MTDs, based upon decreased body weight gain in the 3-mo dose-ranging study and upon decreased body weights (15-20%) in both males and females in this study.

However, it was noted that diminished palatability of the diet due to the presence of drug might have been responsible for the decreased body weights (and food consumption) that limited the doses in this study; and that higher doses/exposures might have been achieved using a different route, such as oral gavage. Nonetheless, there appeared to be a high safety margin for systemic exposure to duloxetine in HD rats compared with that in humans (i.e., ~20-fold for 60 mg/day recommended dosing in humans and ~10-fold the maximum recommended human dose (MRHD) of 60 mg BID). It was also pointed out that dietary administration would better model the human situation, if the half-life in humans was considerably longer than in rats; and this is the case, with a half-life of ~12 hr in humans compared with ~3hr in rats.

After the meeting, the Reviewer found evidence of decreased body weights and food consumption in oral gavage (reproductive toxicity) studies in CD Sprague-Dawley rats using daily doses similar, on a mg/kg basis, to the dietary ones used in the carcinogenicity study (specifically, 45 mg/kg in males and 30 mg/kg in females). A similar effect was also seen in a pharmacology study of meal-fed obese Zucker rats treated with duloxetine by intraperitoneal injection (7.2 mg/kg). Although there is apparently no data to allow a comparison of the systemic exposures achieved at toxic doses by different routes of administration, it seems likely that decreased food consumption and decreased body weight would limit doses regardless of route.

Consequently, the Reviewer now feels that the dietary doses are adequate.

Increased incidence of tumors was limited to a slight increase in the incidence of benign interstitial cell tumor in testes of male rats, a common, benign tumor.

Executive CAC Recommendations and Conclusions:

The Committee agreed with the Sponsor and the Reviewer that the mouse study was adequate and that the positive carcinogenicity findings were hepatocellular adenomas and carcinomas in livers of high-dose females.

The Committee agreed that the rat study was negative for carcinogenicity findings. The Committee felt that the rat study would be considered adequate, if it could be determined that the decreased body weights (and food consumption), which limited the doses, might be due to pharmacologically-mediated appetite suppression rather than diminished palatability. [The evidence (found after the meeting and described above) that administration by oral gavage or intraperitoneal injection also decreased body weights and food consumption should alleviate this concern.]

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

cc:\
/Division File, HFD 120
/ BRosloff, Team leader, HFD-120
/ LFossom, Reviewer, HFD-120
/ DBates, CSO/PM, HFD-120
/ASeifried, HFD-024

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Linda Fossom
9/11/02 11:00:59 AM
PHARMACOLOGIST

Barry Rosloff
9/11/02 11:31:17 AM
PHARMACOLOGIST

9/4/02

NDA 21-427 - SUPERVISORY PHARMACOLOGY MEMO

I concur with the conclusions and recommendations made in Dr. Fossom's superlative review, with the following exceptions:

1. I disagree with the need to perform additional studies on the human metabolites which in general were not well-covered in animals. These are the glucuronide conjugate of 4-OH duloxetine and the sulfate conjugate of 5-OH, 6-MeOH duloxetine (hereafter abbreviated "A" and "B", respectively). These are the major circulating metabolites in humans, estimated to comprise 27 % and 12 %, respectively, of the plasma AUC in humans. (The parent compound is 3 % of the human AUC). My reasons are as follows:

   a. Both metabolites are conjugates; although there are examples of active conjugates, in general conjugation is a detoxification mechanism. (It is noted that these metabolites were inactive in blocking neurotransmitter uptake, although it does not necessarily follow that they are inactive regarding toxicity).

   b. Metabolite A (27 % of human AUC) was present at adequate levels in mouse plasma (50 times human AUC in the carcinogenicity study), and can thus been said to have been adequately evaluated for carcinogenicity (and to some degree for chronic toxicity) in this species. (It was also present in rat plasma, although at relatively low levels, i.e. less than 1/10 human in the carcinogenicity study).

   c. Metabolite B (12 % of human AUC), while not detected in animal plasma, was detected (in both conjugated and free form) in various animal excreta; while this doesn't indicate adequate systemic exposure, it does indicate that at least some duloxetine was metabolized by the same pathway as occurs in humans. (Metabolite A was also present in animal excreta).

   In sum, although it is somewhat disconcerting that so little of the circulating material in humans is well-covered in animals, I do not believe that further studies are necessary for the reasons cited above.

2. I disagree with the need to repeat the *in vitro* chromosomal aberration study. Although current guidelines were not followed as discussed in the review, the weight of evidence from the 6 genotoxicity assays which were performed is that duloxetine is not genotoxic. (However, since it was not optimally performed, I do not think that this study should be included in the labeling).

(I do concur with the recommendation that the sponsor should submit data indicating whether the — 'mpurities discussed in Dr. Fossom's review were present at adequate levels in the pivotal preclinical studies; if they were not, additional studies to qualify them will be required).

/S/
Barry Rosloff

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Barry Rosloff
9/11/02 11:57:28 AM
PHARMACOLOGIST