SEGMENT II REPRODUCTION STUDY IN RATS

A. Method

1. Strain: Long-Evans

2. Dosage: 22 F at 0, 20 at 150, 20 at 300, and 23 at 450 mg/kg/day, by gavage, from days 6-15 of gestation. (Group numbers refer to number of pregnant dams whose offspring were examined for abnormalities; the number which were started on the study cannot be determined from the data presented).

3. Procedure: Dams sacrificed day 20 of gestation, laparotomies performed. All fetuses examined for external malformations, approximately 1/3 for visceral defects (Wilson method), and approximately 2/3 for skeletal defects (Alizarin Red S staining)

B. Results

1. Observed signs in dams

Ataxia, urinary incontinence, and gnawing on cage and/or forepaws seen in 3/28 MD and 24/53 HD.

2. Dam mortality

24/53 HD died on or before day 17 of gestation (one due to dosing accident; 19 exhibited signs as stated above).

3. Bodyweight of dams

Weight loss in all treated groups after first dose, but then caught up and slightly surpassed mean control values.

4. Number of CL and implantations

Slight increase in CL/dam and slight decrease in implantations/dam in all treated groups, not dose related.

5. Number of live and dead fetuses per dam

No drug effects

6. Number of resorbed fetuses per dam

Slight increase in LD and HD, mainly due to 1 dam in each group.

7. Fetal weights

Slight decrease in all drug groups, not dose-related

8. Fetal lengths

Very slight decrease in all groups, not dose related.

9. Fetal abnormalities

a. Number of fetuses examined

	C	LD	MD	HD
gross	209	193	197	220
visceral	70	66	69	77
skeletal	139	127	128	143

b. Gross and visceral abnormalities - no drug effects

c. Skeletal abnormalities

The sponsor concludes that there were no significant treatment effects; however, the incidence tables show a trend toward decreased ossification of several structures at the higher doses, for example:

% with reduced ossification

	C	LD	MD	HD
interparietals	10.1	7.9	14.8	14.0
parietals	12.9	7.2	10.9	16.1
supraoccipital	14.4	11.8	27.3	21.0

The incidence of unossified second sternebrae was increased at LD and HD (5.8%, 19.7%, 8.6% and 22.4% in controls, LD, MD, and HD, respectively). However, the incidence of unossified 5th sternabrae appears to be decreased at MD and HD (about 1/2 control incidence).

The number of total skeletal findings per number of fetuses examined was increased in all treated groups: 3.6, 4.0, 4.2, and 4.3 in controls, LD, MD, and HD, respectively. The number of fetuses with skeletal findings per number of fetuses examined was not affected by drug, in that virtually all fetuses examined had at least one finding.

SEGMENT II REPRODUCTION STUDY IN RABBITS (1 OF 2 STUDIES IN RABBITS)

A. Method

1. Strain: New Zealand White

2. Dosage: 21 F at 0, 22 at 50, 21 at 100, and 24 at 150 mg/kg/day, by gavage, from days 6-18 of gestation.

3. Procedure: Does artifically inseminated. Ovulation induced naturally via mounting by bucks. Does sacrificed day 29 of gestation. All fetuses examined for external malformation, approximately 1/3 for visceral defects (Wilson method), and approximately 2/3 for skeletal defects (Alizarin Red S staining).

B. Results

1. Observed signs in does

a. Slight to severe clonic convulsions in 1/21 MD and 8/24 HD. One HD died during, and 1 MD survived, an opisthotonic convulsion.

b. Number of does failing to eat all of daily food ration (100 g) on 1 or more days: 11/21, 15/22, 16/21, and 24/24 in controls, LD, MD, and HD, respectively.

2. Doe mortality

a. Deaths: 1 control and 2 LD (uncertain cause), 2 MD (dosing accidents), and 1 HD (following opisthotonic convulsion).

b. Sacrifices prior to delivery: 1 LD (broken back), and 2 MD (1 broken back, 1 aborted day 25 of gestation).

3. Bodyweight of does - no drug effect.

4. Number of does pregnant at day 29 of gestation: 17, 18, 16 and 17 in controls, LD, MD, and HD, respectively.

5. Number of abortions, live fetuses/doe, dead + resorbed fetuses, and total implants

No drug effect

6. Mean fetal weight

Slight decrease in all drug groups, dose-related

7. Mean fetal length

Very slight decrease at HD

8. Fetal abnormalities

a. Number of fetuses examined

	C	LD	MD	HD
gross	140	135	114	140
visceral	45	46	37	47
skeletal	95	89	77	93

b. Percent incidence of malformations (number of malformations X 100/number fetuses examined)

	C	LD	MD	HD
1) gross	0	0	0.9	2.1
2) visceral	0	8.7	5.4	6.4
3) skeletal	2.1	5.6	0	6.5
4) percent of does with malformed fetuses of any type	5.9	33.3	12.5	41.2

The sponsor states that although there does appear to be a drug-related increase in malformations, it is not considered biologically significant primarily because no pattern of abnormalities was seen, i.e., except in one instance not more than one fetus per group had any given abnormality, and there was little overlap of types of abnormalities among the different groups. The sponsor also states that all abnormalities seen in the drug groups that were not seen in the concurrent controls have been reported to occur spontaneously.

c. Incidence of common variants

1. Visceral - no drug effect

2. Skeletal

a. The percent of fetuses examined having unilateral or bilateral supernumerary 13th ribs was as follows:

C 26.3
LD 41.6
MD 45.5
HD 55.9

The percent of does with fetuses having this finding was:

C	58.8
LD	72.2
MD	68.8
HD	94.1

The sponsor states that supernumerary ribs are considered to be a normal skeletal variant in rabbits with incidences as high as 75-100%. The increased incidence in the drug groups was considered by the sponsor to be secondary to maternal toxicity.

b. There was a slight increase in incidence of decreased ossification of the palate in all drug groups, not dose-related (8.4, 14.6, 14.3, and 14.0% in controls, LD, MD, and HD, respectively). The incidence of decreased ossification of other bones was not increased by treatment.

SEGMENT II REPRODUCTION STUDY IN RABBITS (1 OF 2 STUDIES IN RABBITS)

(Performed by

A. Method

1. Strain: New Zealand White

2. Dosage: 20 F at 0, 20 at 25, 28 at 50, 20 at 100, and 20 at 150 mg/kg/day, by gavage, from days 6-18 of gestation. (The group numbers refer to the numbers of pregnant does whose offspring were examined for abnormalities; the numbers which were started on the study cannot be determined from the data presented.) Thirteen F of the 50 mg/kg group were accidentally given 100 mg/kg on 1 day.

3. Procedure: Chorionic gonadotropin was given i.v. to stimulate ovulation after mating. Fetuses were delivered by cesarean section on day 29 of gestation. All fetuses were examined for external anomalies, dissected and examined for visceral anomalies, and cleared and stained with Alizarin Red S and examined for skeletal anomalies.

B. Results

1. Observed signs in does

a. LD and MD - no drug effect

b. M-HD - slight hypoactivity

c. HD - slight hypoactivity plus (in 9 does) convulsions, ataxia, tremors, loss of righting reflex, hyperpnea, and muscle spasms.

2. Doe mortality

Number dying or sacrificed due to injury = 0, 1, 1, 2, and 1 in controls, LD, MD, M-HD, and HD, respectively.

3. Bodyweight of does

Weight gain at M-HD and HD less than controls (very slight effect at M-HD)

4. Mean number of CL and implantation sites, and number of live, dead, or resorbed fetuses per doe.

No drug effects

5. Mean fetal weight

Very slight dose-related decrease in all drug groups except LD.

6. Fetal abnormalities

a. Number of fetuses examined:

C	149
LD	146
MD	210
M-HD	142
HD	145

b. Incidence of anomalies

The table on the following page, taken from the IND, shows a trend toward increased incidence of gross, soft-tissue, and skeletal anomalies in the treated groups, which tended to be dose related in some instances.

The increase in gross and soft-tissue anomalies was not statistically different from controls ($\alpha = .05$), and was not discussed by the sponsor. From the table which gives the findings in individual fetuses, it does not appear that there was any pattern showing an increase in any specific anomaly in the treated groups.

Regarding skeletal anomalies, statistically significant drug effects were found for 2 anomalies which are considered common variants: accessory rib(s) (incidence = 29, 49, 51, 48, and 49% in controls, LD, MD, M-HD, and HD, respectively), and delayed ossification of the fifth phalanx of the forelimb (incidence increased at HD only; incidence = 3.4% in controls, and 10.1% at HD). In addition to these findings, a few fetuses in each drug group had "barbell" shaped thoracic centra (incidence = 0, 2.7, 1.0, 0.7, and 4.1% in controls, LD, MD, M-HD, and HD, respectively). The sponsor concludes that these skeletal findings are secondary effects of maternal toxicity in the drug-treated groups.

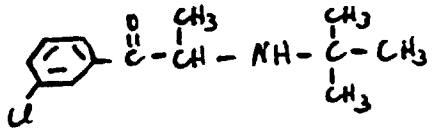
	C	LD	MD	M-HD	HD
No. Fetuses with Gross Anomalies (%)	1 (0.7)	5 (3.4)	5 (2.4)	4 (2.8)	9 (6.1)
No. Fetuses with Soft-tissue Anomalies (%)	4 (2.7)	4 (2.7)	4 (1.9)	6 (4.2)	8 (5.5)
No. Fetuses with Skeletal Anomalies (%)	79 (53.0)	107 (73.3)	135 (64.3)	85 (59.9)	102 (68.9)*
No. Fetuses Showing One or More Gross Anomalies (%)	1 (5.0)	3 (15.0)	2 (7.1)	2 (10.0)	2 (10.0)
No. Fetuses Showing One or More Soft-tissue Anomalies (%)	3 (15.0)	4 (20.0)	4 (14.3)	2 (10.0)	6 (30.0)
No. Litters Showing One or More Skeletal Anomalies (%)	20 (100.0)	19 (95.0)	26 (92.9)	18 (90.0)	20 (100.0)

* Significantly higher than control group (α = .05)

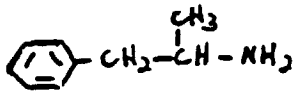
SUMMARY

A. Pharmacology

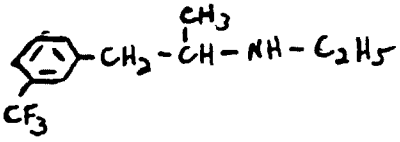
Bupropion (B) is structurally similar to amphetamine, fenfluramine, diethylpropion, and other phenethylamine derivatives.



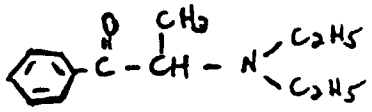
BUPROPION



AMPHETAMINE



FENFLURAMINE



DIETHYLPROPION

Its pharmacological profile is that of a CNS stimulant with several similarities to amphetamine. B was active in 3 types of tests which are predictive of antidepressant activity: prevention or reversal of tetrabenazine/reserpine effects in mouse, decreased immobility in the Porsolt behavioral despair test in rats, and potentiation of the behavioral effects of pargyline + DOPA in mice. In these studies B had an i.p. ED 50% of 10-12 mg/kg (32 mg/kg p.o. in 1 study); activity was sometimes seen at doses as low as 6.5 mg/kg i.p. The potency of B in these tests ranged from 3-5x less potent to equipotent with classical tricyclics. B produced dose-related CNS stimulation in rats and mice as evidenced by increase in locomotor activity as well as by performance in several operant behavioral tests; the potency of B was generally 10-50x less than that of amphetamine in these studies. The ED 50% for increased locomotor activity in mice was approximately 2x that for antitetrabenazine effect whereas the reverse was true for amphetamine and methylphenidate, suggesting a more specific "antidepressant" effect for B. However, some increase in locomotor activity was seen with B at all doses but the lowest which were effective in the antitetrabenazine test. Data in rats further suggested that there is little or no separation of CNS-stimulating and "antidepressant" doses.

B was shown to be a relatively weak blocker of the uptake of NE and 5 HT into brain and peripheral nerve compared with classical tricyclics. It was somewhat more potent in blocking DA uptake although the dose in rat (40 mg/kg i.p.) needed to produce serum levels high enough to cause 50% inhibition of DA (or NE) uptake into brain synaptosomes was 4x greater than the ED 50% in the Porsolt test; thus the blockade of DA (or NE) uptake is probably not involved in the "antidepressant" effect of B. (The reverse holds true for imipramine regarding the potency ratio for "antidepressant effect" and NE uptake blockade.) However, destruction of dopaminergic neurons with 6-hydroxydopamine + DMI blocked the effect of B in the Porsolt test in rats, suggesting that DA neurons are involved in some way.

B did not inhibit MAO or elevate brain NE or DA at relatively high doses.

Many similarities between the pharmacological profiles of B and amphetamine (and other CNS stimulants) were noted,, along with some differences. These may be summarized as follows:

1. B, amphetamine, and methylphenidate all had dose-related antitetrabenazine effects and caused dose-related increases in locomotor activity in mice. However, the ratio of the i.p. ED 50% values for these 2 effects was approximately 1:2 for B and 2:1 for amphetamine and methylphenidate. (In contrast, classical tricyclics cause <u>decreased</u> locomotor activity above "antidepressant" doses.)

2. Amphetamine and methylphenidate reversed tetrabenazine-induced sedation in mice whether given before or after the tetrabenazine; B was only active when given before.

3. Selective depletion of brain DA blocked the locomotor effects of both B and amphetamine; selective depletion of NE had no effect on either drug.

4. The locomotor effect of B and methylphenidate depends primarily on a storage (reserpine-sensitive) pool of catecholamines, whereas that of amphetamine depends primarily on newly synthesized (alpha-methyltyrosine sensitive) catecholamines.

5. B caused an increase in stereotyped behavior in rats; no direct comparison to amphetamine was made.

6. Several behavioral (operant) tests showed the profile of B to be more similar to amphetamine than to classical tricyclics.

7. B had an anorexic effect in mice. (Oral potency at least 2x less than that of fenfluramine and diethylpropion; amphetamine not used.)

8. Drug discrimination studies in rats showed similarities between B and several CNS stimulants (e.g., amphetamine, methylphenidate, caffeine, cocaine) as well as to the newer antidepressants viloxazine and nomifensine.

9. At high doses B caused hypothermia in mice whereas amphetamine caused hyperthermia.

10. Grouping of mice caused an increase in the i.p. lethality of amphetamine but had no effect on that of B; B decreased the lethality of amphetamine in grouped mice.

Cardiovascular studies showed rather large but generally transient decreases in CO and right ventricular contractile force, and both increases and decreases in heart rate and blood pressure, at i.v. doses of 1-20 mg/kg in anesthetized dogs and cats. (It is not clear if these results were corrected for vehicle effects). In conscious dogs, 20 mg/kg p.o. caused slight increases in HR and BP (lasting at least 6 hr.); in conscious rats 50 mg/kg caused a slight increase in HR (lasting 3 hr). Comparison drugs were not used in these studies so that the relative potency of B in causing these changes is not known. No effect on EKG (aside from increased HR) was seen in dogs at 10 mg/kg i.v. (2 mg/kg/min). In dogs, 5-10 mg/kg i.v. caused rather large increases in respiratory rate and smaller increases in minute volume. A relatively weak depressant effect on cardiac tissue in various in vitro preparations was noted which may have been due to the local anesthetic properties of B (equipotent with cocaine in guinea pig cornea); the potency of B was generally 5-15x less than that of imipramine and amitriptyline.

Several studies were performed to assess the anticholinergic effects of B. Effects were generally weak or absent (except for dose-related mydriasis in mice, although it is not clear if this represents an anticholinergic or sympathomimetic effect), and B was significantly less potent than amitriptyline and imipramine when compared. Antagonist actions at other receptors (adrenergic, serotonergic, histaminergic) were generally weak or absent; however reference drugs (which could have validated the systems as well as estimated the relative potency of B) were not used. Likewise, binding studies showed little or no interaction of B with a variety of receptors, but no reference drugs were used.

B. ADME/Pharmacokinetics

ADME/pharmacokinetic studies were performed in rat, mouse, and dog. After oral dosing, plasma levels of B peaked rapidly (within 1/4-1/2 hr) and declined rapidly with a T 1/2 in the 1-4 hr. range. Over a dosage range of 10-100 mg/kg/ p.o. in rats, plasma levels increased with increasing dose but slightly less than proportionately at the highest dose. Studies comparing plasma AUC after i.v. and p.o. dosing showed a bioavailability of 8-21% in rats and 4% in dogs; however, excretion studies using labelled drug showed complete absorption in dogs and a high if not complete degree

of absorption in rats after p.o. dosing. B was widely distributed to tissues in rats; levels were highest in liver and lung after p.o. and i.p. dosing, respectively; lowest levels were in plasma. B was shown to be rapidly and extensively metabolized, which is in agreement with the low oral bioavailability of the drug. Plasma and tissue levels of metabolites were generally substantially higher than those of unchanged drug (exception: brain). Very little unchanged B was found in rat or dog urine; acidic metabolites (m-chlorohippuric and m-chlorobenzoic acids, and a conjugate of the former, were identified), presumably arising from side chain oxidation, were predominant. This is in contrast to human urine, where acidic and basic metabolites were present in nearly equal amounts. Plasma and tissue levels of metabolites declined much more slowly than those of unchanged drug (e.g. in 1 study the plasma T 1/2 for metabolites appeared to be about 12 hours). This suggests that whereas the parent drug is unlikely to accumulate with repeated dosing due to its short T 1/2, metabolites may. However, in one mouse study, 10 days dosing did not lead to an accumulation of metabolites; such a tendency may have been counteracted by an enzyme - induction effect (levels of unchanged B were decreased).

The ability of B to induce liver microsomal metabolic enzymes was demonstrated in rat, mouse, and dog. In rat, pre-treatment with 15-50 mg/kg/day p.o. for 13 days decreased the rise of B in plasma seen after an acute dose of 50 mg/kg i.p., and 50 mg/kg/day p.o. for 4 days decreased the rise of B in tissues seen after an acute dose of 50 mg/kg i.p. In mouse, 50 mg/kg i.p. for 8 or 10 days decreased the rise in whole body level of B seen after an acute dose of 50 mg/kg. In dog, plasma levels after 1 year treatment at 40 or 80 mg/kg/day p.o. were significantly less than those seen on day 1. Studies on pentobarbital sleep time in mice showed a decrease after 5-150 mg/kg/day p.o. for 10 days (effect at HD slightly less than effect of phenobarbital pretreatment at 80 mg/kg/day); in rats a slight decrease was seen at high doses (100-150 mg/kg/day) only. It is possible that part or all of these effects on pentobarbital sleep time were due to CNS stimulation by B; thus while it appears that B can induce its own metabolism, the ability to induce the metabolism of other compounds has not been clearly demonstrated.

Excretion of B + metabolites was shown to be primarily urinary in rats (78%) and dogs (100%).

Over concentration ranges that were stated to be "normally found in animals and during clinical studies in man," B was 75-85% bound to plasma proteins from mouse, rat, dog, and man. Binding was generally constant over the concentration ranges used, although it tended to fall off in man at the highest concentration (1000 μM).

There appears to be some sex differences in the disposition of B, at least in rats. Plasma and tissue levels of unchanged drug, plasma AUC, and oral bioavailability were several-fold greater in F; T 1/2 was greater in F in one rat study but apparently not in another. There did not appear to be

any important sex differences in metabolic pattern or excretion, although data on these points were limited. No sex differences in plasma levels in dogs were apparent although only 2 dogs per sex were used. The acute toxicity of B in rats was slightly greater in F than M, but the reverse appeared to be true in the chronic rat toxicity studies.

C. Toxicology

The acute oral LD 50 was 544 (M) and 636 (F) mg/kg in mouse and 607 (M) and 482 (F) mg/kg in rat. Acute i.p. LD 50 was 273 and 263 mg/kg in male mice and male rats, respectively. Prominent acute signs included: mouse - ataxia,, convulsions, prostration, ptosis, and compulsive gnawing by both routes, plus labored breathing, decreased respiration, and salivation after i.p. only; rat - ataxia, loss of righting reflex, labored breathing, prostration, salivation, ptosis,, arched back, and compulsive gnawing by both routes.

Acute p.o. toxic interaction studies were performed in rats. Phenelzine (at highest no-effect and highest non-lethal doses) caused a marked decrease in the LD 50 of B. (However, no pharmacodynamic interactions were seen in several tests at lower doses). Only a slight potentiation of the lethality of B was caused by treatment with ethanol at its highest non-lethal dose; this was seen in F only. Lethal potentiation was noted between B and amitriptyline (each given at 1/2 LD 50), in F only.

The following oral subacute/chronic toxicity studies were performed (daily dose in mg/kg in parentheses):

1. Rat - 3 month (150, 300, 450)
2. Rat - 55 week (25, 50, 100)
3. Rat - 2 year (100, 200, 300)
4. Mouse - 21-22 month (50, 100, 150)
5. Dog - 3 month (15, 35, 75→150)
6. Dog - 1 year (40, 80, 150)

The principal findings are summarized as follows:

1. Rat

Increased mortality, associated with convulsions, was seen in the 2 year study at all doses (except LD M), and was marked at HD (300 mg/kg). No effect on mortality was seen in the 55 week study (HD = 100); in the 3 month study 2/20 died at 450. Observed signs included urinary incontinence/urine staining (all studies, all doses), dried blood around nose/mouth (55 wk and 2 yr studies, all doses), and convulsions (2 yr study, all doses). Slight decreases in bodyweight gain were seen in all M groups in the 2 yr study. Slight decreases in blood glucose were seen above 100-150 mg/kg. The most prominent post-mortem findings were as follows:

a. Liver

In the two-year study there was an increase in incidence of hyperplastic nodules and hepatocellular hypertrophy at all doses; hyperplasia was increased at LD and MD only. (In a consultant report many of the hyperplastic nodules were reclassified as "foci or areas of altered hepatocytes"). The incidence of these findings is underestimated in the drug groups in a dose-related fashion due to the increased mortality and the late appearances of the lesion. (Most hyperplastic nodules were found at the terminal sacrifice, and almost all were found after 90 weeks). There was no increase in the incidence of hepatocellular carcinoma; the observed incidence (0/147, 3/140, 1/141, and 1/123 in control, LD, MD,, and HD, respectively) is within the historical control range. Similar findings were not seen in the 55 week study (HD = 100); in the 3 month study a low incidence of hyperplasia and "prominent cellular organelles" was seen at all doses. Increased liver weights were seen in all studies at all doses except LD in the 3 month study. Grossly, in the 2 yr. study slight increases in the incidence of masses/nodules/raised area (F only) and dark red/brown/hemorrhagic foci were seen at all doses at termination but not among deaths. The sponsor suggests that these proliferative changes in liver may represent either (1) an indication of microsomal enzyme induction or (2) an adaptive response to hepatic injury. Regarding the former, B has been shown to induce its own metabolism (see above). Regarding the latter, no other indication of hepatic damage (including blood chemistry) was obtained in rats, although some indications of liver damage were obtained in dogs.

b. Hemosiderosis

In the 55 week study an increase in hemosiderosis (as determined either by H + E stain, iron stain, or presence of pigment-containing macrophages) was seen in spleen, kidney, lung, and liver. This was seen primarily at HD, although lower doses were not examined in lung and liver. Likewise, in the two year study, evidence of increased hemosiderosis was seen in spleen, lung, and lymph nodes at MD and HD. (LD not examined in these organs). No other pathological findings were present to help explain the increased hemosiderosis. Hematology did not reveal any striking abnormalities. (2/20 HD in the 55 week study had low Hb, Hct, and RBC; no effect in two year study; slight decreases in Hb and Hct seen in the 3 month study but no hemosiderosis reported).

c. Kidney

Slight increases in the incidence of chronic nephritis were seen in the 55 week study (HD only) and in the two-year study (MD and HD; LD not examined). There were no consistent effects on lab tests indicative of renal function; in the 55 week study there were elevations of BUN in 3 of 40 rats at MD and HD. Kidney weights were elevated in all studies at all doses.

d. Neoplasia

There were no drug-related increases.

2. Mouse

In the 22 month study mortality was increased in all M groups and HD F. There was no effect on weight gain. As in rats, convulsions were seen (MD and HD). Laboratory studies were not performed. The most prominent postmortem findings were seen in uterus, consisting of a dose-related increased incidence of extremely dilated blood vessels, with thrombus, in all F groups. This increase was seen both among mice which died and those which survived to termination, suggesting a lack of association with lethality. Grossly there was an increased incidence of uterine nodules/masses; according to the text these were actually extremely dilated veins with thrombosis. The red urogenital staining noticed during the in-life phase was probably also related to these changes. Also seen in uterus was increased incidence of acute metritis/pyometritis in all drug groups (dose-related) and slightly increased incidence of uterine hemorrhage in all drug groups (not dose-related). Splenomegaly and hematopoiesis in spleen and liver were also seen in F; the pathology report considered these to be secondary to the uterine blood loss although an independent analysis by the sponsor did not show a good correlation between the uterine and spleen/liver changes. Changes similar to those in uterus were not clearly seen in other organs, although a low incidence of hemorrhage and ulcer in stomach and small intestine was noted, primarily at HD, the incidence of thrombus in heart was increased in HD among deaths but not at termination, and the incidence of congestion/hemorrhage in lung was increased in HD M. An increased incidence of atrophic tubules in testes at HD was also seen in this study, although there was no effect on the incidence of aspermatogenesis. As in the rat study, there was no effect on the incidence of neoplastic changes.

3. Dog

No significant toxic effects were seen in the 90 day study (HD = 75→150); a slight increase in liver weight was seen with no associated pathology. In the 1 year study the HD (150) produced 3/16 deaths. This dose also produced convulsions in 1 dog and body trembling in

several dogs. Emesis and ptyalism were seen at both MD and HD. Bodyweight gain was decr eased at HD. Values for RBC, Hb, and Hct tended to be decreased at the higher doses but this did not progress with time and no effect was seen in recovery dogs. (Slight decreases in Hb and Hct were also seen in the 90 day study.) There was a dose-related elevation of serum alkaline phosphatase in all groups at all months measured, and the magnitude increased over time; no effect seen in recovery dogs. Elevations of SGOT and SGPT were also seen mainly at the higher doses, starting at 3 months but not clearly progressive over time. Some recovery dogs still had elevated SGPT after the recovery period, but of smaller magnitude. Slight increases in BSP retention were seen at MD and HD. Liver weights were increased in all groups (dose-related) at both 6 and 12 months but not at recovery. Microscopic exam of liver showed several drug-related changes including finely granular "ground glass" cytoplasm (MD and HD, seen at 12 months but not at 6 months or after recovery period), dark brown pigment in hepatocytes and phagocytic cells (MD and HD, seen at 12 but not at 6 months, and seen at all doses after recovery period), slight coarse vacuolation of hepatocytes (seen in HD at 12 months and in LD and MD at 6 months and in the 1 MD which died; not seen after recovery period), and bile duct proliferation (very slight to slight) (seen at MD and HD at 6 months and at HD at 12 months, also seen after recovery period in 2/4 LD and 2/3 HD but also in 1/4 control and 0/3 at MD). Kidney weight was elevated in all groups at 6 months; however at 12 months an increased relative weight only was seen (MD and HD only). Histologically, the incidence and severity of brown pigment in tubular epithelium was increased in all groups at termination. The incidence of this finding among recovery dogs was similar across groups although severity was greater at MD and HD. No clear abnormalities of renal function were seen in this study.

D. Mutagenicity

B was weakly positive in some Salmonella strains in the Ames Test. (TA 100, both with and without metabolic activation, and TA 1535, with activation only). Greatest effects were 2-3 x control revertant count; positive controls caused 6-10 x increases. In a rat bone marrow chromosome study, an increase in aberrations was seen at 300 but not 100-200 mg/kg p.o., given for 5 days; the increase was 2-3 x control compared to 6-19 x for the positive control. In a study of binding to rat liver DNA after oral administration, it was found that the binding of bupropion (+ metabolites) was much lower than that of known hepatocarcinogens and was concluded to be nonspecific.

E. Reproduction

A 2 generation reproduction and fertility study was performed in rats. Both M and F (of the F0 generation only) were drug treated, dosages = 100, 200, and 300 mg/kg/day. Except for wobbly gait in 1 MD and 1 HD, no

drug-related signs were observed. Body weight gain was slightly increased in all treated groups, but not dose-related. There was no drug-related increase in mortality. No drug effects on M or F mating performance, on F fertility or reproductive parameters, or on pup (F 1 generation) survival and body weight were seen. There were no significant effects on mating or reproductive performance of the F 1 generation. Pup survival (F 2 generation) was not affected by treatment, although F 2 pups in all drug groups had slightly increased body weight.

Segment II reproduction studies were performed in rats and rabbits. In rats (dosages = 150, 300, and 450 mg/kg/day), signs including ataxia, urinary incontinence, and gnawing were seen primarily in HD dams. Mortality was increased at HD (24/53 = 45%). Bodyweights were transiently decreased in all drug groups after the first dose. There was a slight decrease in fetal weight and length in all drug groups, not dose-related. There were no drug-related effects on gross or visceral fetal abnormalities. A slightly increased incidence of reduced or absent ossification of some bones was seen at MD and HD and to a smaller extent at HD; this was reflected in a slight increase in total skeletal findings in all drug groups.

LD

Two segment II reproduction studies were performed in rabbits, 1 by the sponsor's lab and 1 by
Corporation. Dosages were 25 (latter study only), 50, 100, and 150 mg/kg/day. Does displayed slight hypoactivity at 100 and 150 mg/kg, and convulsions, ataxia, tremors, and hyperpnea were seen in some does at 150 (convulsions also seen in 1 doe at 100 mg/kg). In one study, decreased ~~dose~~ doe food consumption on individual days was seen in all drug groups, but no overall effects on body weight were seen; however, the other study showed reduced weight gain at 100 and 150 mg/kg. There was no drug-related increase in mortality in either study. Fetal weight was slightly reduced in all drug groups at 50 mg/kg and above, dose-related. Fetal length (reported in 1 study) was slightly reduced at 150 mg/kg. There was a trend toward an increase in gross, visceral, and skeletal abnormalities in fetuses of all drug groups, which was partly dose-related. The increase in gross and visceral abnormalities does not appear to be biologically significant in that no pattern of abnormalities was seen, i.e. there was no significant increase in any particular type of abnormality, and the overall percent of fetuses affected was relatively low. Regarding skeletal abnormalities, a significant increase in supernumerary ribs occurred in all drug groups which was dose-related in 1 study but not in another. In addition, one study showed an increase in reduced ossification of the palate (all drug groups, not dose-related), and the other study showed an increase in delayed ossification of the 5th phalanx of the forelimb at HD only as well as a low incidence of barbell shaped thoracic centra in all drug groups. Reduced ossification (also seen in rat study) and supernumerary ribs are considered to be normal variations, and it is concluded that the above skeletal findings, as well as the findings of decreased fetal weight and length, were secondary consequences of maternal toxicity.

EVALUATION:

The preclinical data submitted for bupropion (B) adequately characterizes the pharmacological and toxicological profile of this drug.

B, unlike classical antidepressants, is a CNS stimulant in animals, with a potency of about 1/50-1/10 that of amphetamine in causing CNS stimulation in various tests in mice and rats. Although it was shown that, unlike for amphetamine, the ED 50% for increased locomotor activity in mice was greater than that for an "antidepressant" (i.e. antitetrabenazine) effect, it appeared that there was acutally little separation of doses producing "antidepressant" effects from those producing at least some CNS stimulation. Several pharmacological similarities between B and amphetamine (and other CNS stimulants) were noted, including self-administration by monkeys, raising the question of possible abuse. Studies addressing this question have been conducted in man.

The sponsor claims that B has less anticholinergic and adverse cardiovascular effects than classical antidepressants. The animal data appear to support the former: in several tests anticholinergic activity was generally weak or absent (except for dose-related mydriasis in mice, although this may represent a sympathomimetic effect) and B was significantly less potent than imipramine and amitriptyline when compared. Regarding adverse cardiovascular effects, however, the relative potency of B and classical antidepressants is difficult to determine based on the data presented. Acute i.v. doses of 1-20 mg/kg in anesthetized cats and dogs produced large but generally transient effects on blood pressure, heart rate, cardiac output, and right ventricular contractile force. In conscious dogs, 20 mg/kg p.o. caused slight increases in blood pressure and heart rate, and in conscious rats 50 mg/kg p.o. caused a slight increase in heart rate. No adverse EKG effects were seen either with a slow i.v. infusion of up to 10 mg/kg in anesthetized dogs or in a 1 year toxicity study in dogs with a maximum daily dose of 150 mg/kg p.o. However, comparison drugs were not used in these studies; a more informative study would have used other antidepressants, and pushed up doses until adverse EKG/cardiovascular effects were seen so that the relative potency of B could be estimated. (In *in vitro* studies, B was approximately 5-15 x less potent than imipramine or amitriptyline regarding cardiodepressant effects but this cannot be readily extrapolated to *in vivo* conditions). The sponsor points out that doses and concentrations causing cardiovascular effects in the above studies were 10-100 x greater than clinically therapeutic plasma levels (and plasma levels in mice after administration of the "antidepressant" ED 50); however this does not address the question of possible overdose effects.

Pharmacokinetic studies showed a sex difference in rats, i.e. higher blood and tissue levels were found in females. (The acute toxicity of B in rats is greater in F than M, although this did not appear to be true regarding chronic toxicity). The drug appears to undergo extensive first-pass

metabolism in animals and has a short T 1/2 (1-4 hr., but much longer for metabolites), however human studies have shown a longer T 1/2 of 14 hr. B was shown to induce its own metabolism, presumably VIA an effect on liver microsomal enzymes, in rat, mouse, and dog.

Segment II reproduction studies, performed in rats and rabbits, showed a tendency toward delayed ossifications and supernumerary ribs, findings which are relatively common in drug studies and are thought to result from maternal stress/toxicity and/or fetal immaturity (although it should be noted that some of these skeletal findings were seen at the lower doses which did not clearly induce other overt toxic effects). In the rabbit studies overall gross and visceral abnormalities were increased at all doses, although the percent of fetuses affected was relatively low and no increase in any specific type of abnormality was seen. A consultant report by Dr. James G. Wilson, an acknowledged expert in teratology, concluded that there were "no major indications of embryo-fetotoxicity observed ... at any dosage."

Carcinogenesis studies in rats (23-24 months) and mice (21-22 months) did not reveal any increase in neoplasia (maximum daily dose = 300 and 150 mg/kg, resp.), despite the fact that B was weakly positive in some strains in the Ames Test and at 300 mg/kg in a rat bone marrow chromosome study.

The major toxicological findings having possible implication for man were the following:

1) Convulsions - seen in rat, mouse, dog, and rabbit, primarily at the higher doses. Studies in rats suggested that chlordiazepoxide was the most effective antagonist of B-induced seizures. (Other benzodiazepines were not tested).

2) Hyperplastic nodules in liver

These were seen in the 2 yr rat (but not mouse) study. (In the 1 year dog study, "ground glass" and vacuolated cytoplasm were seen but it is not clear if these were similar to "foci of cellular alteration" which have been described in rats and have been considered to be related to [possible precursors of ?] hyperplastic nodules). Nodules were seen at all doses (100, 200, 300 mg/kg) in the 2 year rat study, and thus no "no-effect" dose was established. (Nodules were not seen at 25, 50, or 100 mg/kg in the 55 week rat study; however, they did not appear until around 90 weeks in the 2 year study, and thus might have possibly developed at these lower doses had the rats lived long enough). (For comparison, acute ED 50% for antidepressant activity was approximately 10 mg/kg i.p. in rats, and 12.5 mg/kg i.p. and 32 mg/kg p.o. in mice). The toxicological implications of hyperplastic nodules are not clear. The sponsor suggests that they may represent either (1) a reflection of liver microsomal enzyme induction or (2) an adaptive response to hepatic injury. Regarding the former, B has been shown to induce its own metabolism although the ability of B to induce the metabolism of other compounds was not clearly demonstrated. Regarding the latter, no other indication of hepatic damage (including blood chemistry) was seen in rats, although some indications of liver damage were obtained in dogs. There has been much controversy in recent years concerning the possible role of hyperplastic nodules in the development of hepatocarcinomas. Several years ago some pathologists suggested that such lesions should be considered "pre-neoplastic" or "neoplastic" in that it was hypothesized that they could progress to malignant tumors (Squire and Levitt, Cancer Res. 35: 3214, 1975; Williams, Biochem. et Biophys. Acta 605: 167, 1980). These pathologists thus suggested replacing the term "hyperplastic nodule" with "neoplastic nodule". (It has also been suggested that "foci of cellular alteration" are also "pre-neoplastic" in the sense that they might progress to neoplastic nodules or possibly directly to malignant tumors). However, this area was recently the subject of a symposium (Rodent Liver Nodules - Significance to Human Cancer Risk?, Int'l Symposium of the Society of Toxicologic Pathologists, May 10-12, 1982, Reston, VA; proceedings to be published) at which these

earlier hypotheses were challenged. Data was presented showing that in several cases nodules and foci of cellular alteration regressed after cessation of treatment, suggesting that they are not necessarily on an irreversible pathway to malignancy. It has also been shown that nodules are not necessarily transplantable. Based on these observations of a lack of autonomy of these proliferative lesions, an informal consensus was reached agreeing with the proposition that the term "neoplastic nodule" was a misnomer and that such lesions do not necessarily progress irretrievably to malignancy. However, even though these proliferative lesions may not be autonomous and do not necessarily progress irretrievably to malignancy, it is possible that they would progress under the influence of continued drug administration, or alternatively they may simply be "markers" for malignancy (i.e. if a drug produces such lesions it is an indication that the drug is also likely to produce malignancies). Again, based on an informal vote a majority of pathologists present at this symposium believed that the production of nodules or foci of cellular alteration in liver by a chemical was not sufficient evidence to establish that chemical as a hepatocarcinogen. Although several potent hepatocarcinogens have been shown to produce nodules and foci of cellular alteration, several examples were given of chemicals, dietary regimens, and surgical manipulations which produced nodules or foci but did not produce malignancies despite prolonged treatment. (B would also be an example of this). In summary, I believe that given the state of the art in this area, there is not enough evidence at this time to label B a hepatocarcinogen. It is not known if the proliferative lesions produced by B were autonomous since no studies on reversibility or transplantability were performed. However, these lesions did not progress to malignancy despite continued drug administration in a lifetime rat study. In addition, the nodules were very late appearing (most seen at terminal sacrifice; almost all after 90 weeks), in contrast to the effects of established hepatocarcinogens. On the other hand, in view of the still lingering uncertainty in this area (as well as the weakly positive mutagenicity results), I believe that the findings of an increase in proliferative lesions in liver should be mentioned prominently in the labeling, at least until the issue of the carcinogenic significance of these lesions is resolved.

3) A marked toxic interaction between B and phenelzine was demonstrated in rats (although no pharmacodynamic interactions were seen in several tests at lower doses). Thus, as with classical tricyclic antidepressants, the combined use of B and MAO inhibitors in man should proceed cautiously if at all.

Other findings of more questionable or unknown significance to man were the following:

1) Increased hemosiderosis in several organs in the 55 week and 2 year rat studies, primarily at the higher doses. No other pathological findings were present to explain this; RBC, Hct, and Hb were decreased in 2 of 20 HD rats in the 55 week study but no effect seen in the 2 year study; a slight decrease in Hb and Hct was seen in the 3 month study but no hemosiderosis was reported. (A dark brown pigment was seen in liver and kidney in the 1 year dog study and in kidney in the 2 year mouse study; the pigment was not characterized; decreases in RBC, Hct, and Hb were seen in dogs).

2) Increased incidence of extremely dilated uterine blood vessels with thrombus, uterine bleeding, and acute metritis/pyometritis seen at all doses in the 2 year mouse study. It was considered to be an accentuation of a spontaneous lesion; time of onset was not affected. Similar findings were not seen in other species.

3) Some evidence of liver toxicity was seen at all doses in the 1 year dog study, including elevated AP, SGOT, SGPT, and BSP retention. Liver histopathology included findings of pigment, vacuolation, "ground glass" cytoplasm, and bile duct proliferation; necrosis was not reported. Most of these changes were reversible upon cessation of treatment.

RECOMMENDATIONS:

This NDA is approvable based on the preclinical data submitted, with the following recommendations:

1) Regarding the 2 year rat and mouse carcinogenicity studies, the sponsor should indicate how often the drug solutions for dosing were prepared, and what the drug stability was under these conditions, in order to assure that the actual doses administered were what they were stated to have been.

2) The findings in uterus in the 2 year mouse study (extremely dilated blood vessels with thrombus, bleeding, metritis/pyometritis) should be brought to the attention of the clinical reviewer. The relevance of this finding, seen only in mouse, to man is not clear; a review of adverse reactions in this area would be helpful. Post-marketing monitoring as well as inclusion of the mouse results in the labeling should also be considered.

3) The clinical reviewer should consider making combined use of Wellbutrin and monoamine oxidase inhibitors a contraindication (as with other antidepressants) in view of the toxic potentiation noted in rats. (The sponsor mentions this finding in the "Drug Interactions" section of the labeling).

4) The following recommendations concern the proposed labeling:

   a) Carcinogenesis section

      The word "small" in the sentence "In rats there was a small increase in nodular proliferative lesions of the liver..." should be eliminated. The incidence of this finding in the drug groups was several times that in controls.

   b) Mutagenesis section

      The weakly positive effects noted in the Ames Test should be mentioned.

   c) Pregnancy section

      The findings of an increase in fetal anomalies (aside from supernumerary ribs and delayed ossifications) in the rabbit studies should be mentioned. Although there was no strong evidence that the drug was teratogenic (i.e. there was no increase in any specific type of anomaly and the number of fetuses affected was relatively small), the data do raise a suspicion which should be mentioned.

d) Overdosage section

Regarding the last sentence which states that animal data suggest that phenytoin is less satisfactory for treating seizures and barbiturates may worsen seizures: According to the data submitted, phenytoin was not tested for antagonism of seizures (and was completely inactive in preventing seizures at the dosage used) and phenobarbital was active (i.e. shortened seizure time) but less potent than chlordiazepoxide. (Phenobarbital and one dose of chlordiazepoxide apparently increased the number of deaths, but the interpretation of this was not made clear). The sponsor should address this discrepancy and either alter the labeling accordingly or else better explain the results obtained and/or supply additional data which supports the statements made.

Barry N. Rosloff, Ph.D.

Barry N. Rosloff, Ph.D.

cc:
NDA Orig.
HFD-120
HFD-120/BRosloff/7/12/82;8/18/82
HFD-120/JContrera/RDInit. 7/12/82
HFD-180
FT/lca:cpb:7/31/81;8/18/82/Doc.#3245B

APPENDIX 2

Barry N. Rosloff, Ph.D.
October 29, 1982

Pharmacologist Review of INDs (Progress Report of 10/15/82 and NDA 18-644 (Amendment of 11/10/82)

SPONSOR: Burroughs Wellcome Co.
3030 Cornwallis Road
Research Triangle Park, N.C. 27709

DRUG: bupropion HCl
(Trade name: Wellbutrin)

CATEGORY: antidepressant

PREVIOUS PHARMACOLOGIST REVIEW: Original Summary of NDA 18-644 (6/15/82)

NEW PRECLINICAL STUDIES:

1) Acute oral toxicity in neonatal, immature, and adult rats
2) Cytogenetic study in rats

ACUTE P.O. TOXICITY IN RATS:

(Strain: Charles River CD)

AGE OF RATS (Days)	13-14 Day LD 50 (mg/kg)		
	M	F	
6	469	499	
17	739	763	
27	678	>666	(no deaths at this dose)
39	683	342	

Toxic Signs:

1) 6 days old - dose range = 150-450 mg/kg; no signs seen; most deaths occurred on day of dosing

2) 17 days old - dose range = 300-600 ; signs = decreased activity and ataxia (at 400-600); most deaths occurred on day of dosing

3) 27 days old - dose range = 200-500; signs (seen at most doses) = salivation, ataxia, preconvulsive seizures, clonic convulsions (at 400+), loss of righting reflex, prostration, ptosis, gnawing; deaths occurred on day of dosing through 3 days post-dosing

4) 39 days old - dose range = 550-850; signs (seen at most doses) similar to those seen at 27 days (above) plus clonic and/or tonic convulsions (at 650+) and labored breathing; deaths occurred on day of dosing through 3 days post-dosing, with most of the later deaths occurring in F

CYTOGENETIC STUDY IN RATS

A) Dosage

12 M + 12 F at 0, 125, 250, or 500 mg/kg by gavage (single dose). Four/sex/dose sacrificed at 3, 24, or 48 hrs. for bone marrow cell chromosome examination. Positive control = 4 M + 4 F at 0.4 mg/kg triethylenemelamine i.p. (sacrificed at 24 hr.)

Strain = Charles River CD

B) Results

There was no effect of bupropion on chromosomal aberrations.

SUMMARY AND EVALUATION:

The acute oral toxicity of bupropion in Charles River CD rats was greater in animals 6 days old compared with animals 17, 27, or 39 days old, with the exception of day 39 females in which the LD 50 was 1/2 that of 39 day males. (Toxic signs on the day of dosing in 39 day aimals were similar in M and F at the same doses; the excess mortality in F appears to be due primarily to delayed deaths, i.e. occurring days 2 and 3 post-dosing). The results obtained in 39 day old rats (including the sex difference) are in the same ballpark as those previously obtained in 37 day old Long-Evans rats (LD 50 = 607 and 482 mg/kg in M and F, resp.). Sex differences were not apparent in the younger (days 6-27) rats.

Bupropion in acute does up to 500 mg/kg p.o. did not induce chromosomal aberrations in Charles River CD rats. However, a previous study (see Original Pharmacologist Summary of NDA 18-644, 6/15/82) in the same strain showed a slight increase in aberrations at 300 (but not 100-200) mg/kg p.o. given daily for 5 days. Bupropion was also weakly positive in some strains in the Ames Test, but was not strongly bound to rat liver DNA. It also produced an increase in rat liver hyperplastic nodules without increasing hepatocarcinoma in a 2 year rat study (see above-mentioned review for complete discussion). The results in the present submission add a small piece of negative data to a mixed picture regarding the mutagenicity of bupropion; however a more useful study would have been to give the drug subacutely to see if the previous results could be replicated.

RECOMMENDATIONS: None

Barry N. Rosloff

Barry N. Rosloff, Ph.D.

cc:
IND 7,066
IND 13,845
NDA 18-644
HFN-120
HFN-120/BRosloff/10/29/82
RDinit JContrera/11/1/82
HFN-180
F/T: lca:11-3-82
Doc 4158B

APPENDIX 3

Barry N. Rosloff, Ph.D.
9/29/94

Pharmacologist Review of NDA 20-358
Original Summary

SPONSOR: Burroughs Wellcome Co.
3030 Cornwallis Road
Research Triangle Park, NC 27709

DRUG: bupropion (Wellbutrin®) sustained release tablets

CATEGORY: antidepressant

RELATED NDA: NDA 18-644 (NDA for marketed immediate release formulation, same sponsor as present).

NEW PRECLINICAL STUDIES SUBMITTED:

(N.B. These studies were performed as bridging studies to support the sustained release formulation; see "Summary" section for details).

1) 90 day p.o. toxicity in rats.

- Performed by sponsor
- BIN numbers IN2730 and 3M2498A for degraded and undegraded drug, resp.

2) Segment II reproduction in rats

- Performed by Research Triangle Institute
  Research Triangle Park, NC 27709-2194
- BIN numbers 3U6028A, B, and C for degraded drug and 3U6029 for undegraded drug.

3) Ames tests

- Performed by

- BIN numbers used not stated; analysis sheets give BIN numbers 3X6011 A-F for "Wellbutrin plus degradants" but is not clear if these were used in the studies.

4) In vivo cytogenetic study in rats

- Performed by sponsor and by

- BIN number 3U6007E (not clear if this applies to degraded or undegraded drug).

TABLE OF CONTENTS

90 DAY P.O. TOXICITY IN RATS

A) Dosage

Doses and numbers of animals shown below. Note that 3 dose levels of "degraded" drug were used (see Summary section for amounts of degradants) along with one dose level of undegraded drug (hereafter referred to as HD*, equal to the HD of degraded drug). Note 4/sex/dose were used for a 14 day recovery period. Dosing was by gavage.

Strain: Charles River CD

GROUP NO.	SUBSTANCE	DOSE (MG/KG/DAY)	TOTAL NO.[a] OF ANIMALS (MALE/FEMALE)	TOX STUDY UAIN (93-PREFIX)	
				MALE	FEMALE
1	Sterile Water	0	14/14	6025-6038	6150-6163
2	323U66 HCl[b]	75	14/14	6039-6052	6164-6177
3	323U66 HCl[b]	150	14/14	6053-6066	6178-6191
4	323U66 HCl[b]	300	14/14	6067-6080	6192-6205
5	323U66 HCl	300	14/14	6081, 6143, 6083, 6084, 6144[d], 6086-6088, 6145[d], 6090, 6146[d], 6092-6094	6206-6219
6[c]	323U66 HCl[b]	75	12/12	6095-6106	6220-6231
7[c]	323U66 HCl[b]	150	12/12	6107-6118	6232-6243
8[c]	323U66 HCl[b]	300	12/12	6119-6130	6244-6255
9[c]	323U66 HCl	300	12/12	6131-6142	6256-6267

[a] Four males and four females in groups 1-5 were assigned to a 14-day postdose recovery period.

[b] Degraded 323U66 HCl

[c] Groups 6-9 for drug plasma level determinations. Only body weights and observations for clinical signs of toxicity were evaluated.

[d] Replacement animals on dose day 4.

B) Results

1) Observed signs

D-R postdosing salivation at all doses; it was stated that there was no difference between HD and HD* "with respect to incidence or frequency of occurrence".

2) Mortality

Results summarized on attached page. There was an excess of deaths at HD*; however, most of these were "confirmed to be caused by dosing accidents".

The incidence of mortality for all groups is summarized as follows:

Dose (mg/kg/day)	N (M/F)	Number Dead	
		Male	Female
0	14/14	0	1
75[a]	26/26	3	0
150[a]	26/26	0	1
300[a]	26/26	3	1
300	26/26	5	7

[a] Degraded 323U66 HCl
N=Number

The cause of death was determined on the basis of the gross and/or histopathologic findings and is summarized in the chart below:

Group/ Sex	Dose (mg/kg/day)	Animal Number	Day of Death	Cause of Death
2M	75[a]		7	Dosing Accident
			33	Dosing Accident
4M	300[a]		6	Undetermined
			7	Undetermined
5M	300		36	Undetermined
			35	Dosing Accident
			58	Dosing Accident
6M	75[a]		62	Dosing Accident
8M	300[a]		15	Dosing Accident
9M	300		63	Dosing Accident
			39	Dosing Accident
1F	0		38	Dosing Accident
3F	150[a]		35	Undetermined
4F	300[a]		48	Bleeding stomach ulcers
5F	300		40	Dosing Accident
			39	Dosing Accident
			86	Dosing Accident
			40	Dosing Accident
9F	300		40	Dosing Accident
			43	Dosing Accident
			56	Dosing Accident

[a] Degraded 323U66 HCl

3) Bodyweight

No drug effects

4) Food consumption

No drug effects

5) Ophthalmoscopic exam

(Done pre-study, at termination, and after recovery)
No drug effects

6) Hematology

(Done pre-study, wks 7 and 13, and wk 2 of recovery period)

a) WBC were increased in MD F and both HD F groups, not D-R, at 7 and 13 weeks (mean ~ 1.5 x control) but not during recovery period. This was primarily due to increased numbers of lymphocytes.

b) RBC, Hb, and Hct were equivocally very slightly decreased in all F groups, not D-R, at week 13 and during recovery period.

c) Other parameters measured: Rest of WBC differential, platelets, reticulocytes, RDW.

7) Blood Chemistry

(Done pre-study, wks 7 and 13, and wk 2 of recovery period.)

No clear drug effects. Total protein and albumin tended to be very slightly increased in all groups but LDF, not D-R.

Other parameters measured: ALT, AST, AP, total bilirubin, BUN, creatinine, Na, K, glucose, globulin (by subtraction).

8) Urinalysis

(Done in 4/sex/dose pre-study, wks. 7 and 13, and wk 2 of recovery period).

The sponsor states no drug effect; however the small N used makes drug effects difficult to detect.

9) Organ weights

a) Liver

Absolute and relative weights increased at all doses, D-R. (Mean rel. wt. in HD groups approx 1.4 x control. No clear overall difference between HD and HD*). Increase still seen after recovery period (except at LD); the increase in F after recovery period was slightly smaller than that seen at termination, but N too small to conclude this confidently.

b) Thyroid

Slight increase in abs. and rel. wt in both HD M groups. (The effect at HD* was equivocally greater than that at HD; mean rel. wt. 1.29 and 1.13 x control, resp.). No clear effect after recovery, but note small N.

c) Adrenal

Slight increase in abs. and rel. wt in both HD M groups (Mean rel. wt. approx. 1.3 x control; no clear difference between HD and HD*). No clear effect after recovery, but note small N.

d) Kidney

Abs. and rel. wt. equivocally and slightly (mean rel. wt. about 1.2 x control) increased in MD M and both HD M groups; not D-R; equivocal increases also seen after recovery.

10) Gross pathology

No drug effects

11) Microscopic pathology

(Routine exam done in controls and both HD groups at termination, and in all animals which died or were prematurely sacrificed. Among recovery animals, only male kidney, in the control and both HD groups only, was examined.

a) Liver

Increased incidence of hepatocellular hypertrophy, with incidence as follows:

	C	HD	HD*
TERMINATION	0/19	16/17	12/13
DEATHS/SAC.	0/1	3/3	3/7

This finding was not seen in the 2 LD found dead and 1 MD prematurely sacrificed which were examined (but note these exams occurred relatively early in the treatment period). It was described as "minimal; centrilobular" in all animals. Liver was not examined in recovery animals.

b) Kidney

Increased incidence of chronic progressive nephrosis in males, with incidence as follows:

	C	HD	HD*
TERMINATION	3/10	6/8	6/7
DEATHS/SAC	0/0	0/2	3/3
RECOVERY	1/4	4/4	4/4

It was not found among the 2 LD M found dead (but note these deaths occurred relatively early in the treatment period). The severity ranged from very minimal to mild.

12) PK data

Plasma levels of bupropion and 3 metabolites (306U73, 494U73, and 17U67) were assayed pre-dosing and at 0.5, 1, 2, and 4 hr. post-dosing on days 10 and 82 in 3 (sometimes 2)/sex/group/time point. (Exception = the 1 hr. point not done day 82). Results for bupropion and 306U73 are summarized in the attached 2 tables (day 10 and 82, resp.) (Compound 494U73 was present in only a few samples. Values for 17U67 were not given although it was stated that, as for 494U73, the data for 17U67 were insufficient to permit calculation of PK parameters.) From the tables it would appear that:

1) Plasma levels (of both parent and metabolite) were generally not dose-related (except perhaps in F on day 82).

Table 8. Pharmacokinetic Parameters for Bupropion and 306U73 in Rats on Day 10 Following an Oral Administration of Bupropion (TOX 648)

		Bupropion			306U73		
	Gender	AUC (ng/mL·hr)	C_{max} (ng/mL)	T_{max} (hr)	AUC (ng/mL·hr)	C_{max} (ng/mL)	T_{max} (hr)
GROUP 6 75 mg/kg/day (with impurities)	males	287	279	1.0	258	162	1.0
	females	2102	1365	1.0	825	340	1.0
GROUP 7 150 mg/kg/day (with impurities)	males	432	382	1.0	452	301	1.0
	females	2626	2811	1.0	958	398	1.0
GROUP 8 300 mg/kg/day (with impurities)	males	335	266	1.0	666	277	1.0
	females	2839	2181	1.0	3026	1707	1.0
GROUP 9 300 mg/kg/day (without impurities)	males	282	137	1.0	492	236	1.0
	females	2084	1182	1.0	1574	745	1.0

Table 9. Pharmacokinetic Parameters for Bupropion and 306U73 in Rats on Day 82 Following an Oral Administration of Bupropion (TOX 648)

	Gender	Bupropion			306U73		
		AUC (ng/mL·hr)	C_{max} (ng/mL)	T_{max} (hr)	AUC (ng/mL·hr)	C_{max} (ng/mL)	T_{max} (hr)
GROUP 6							
75 mg/kg/day	males	966	545	0.5	325	133	0.5
(with impurities)							
	females	2469	1032	0.5	473	159	0.5
GROUP 7							
150 mg/kg/day	males	1410	1001	0.5	415	186	0.5
(with impurities)							
	females	3443	1529	0.5	613	194	4
GROUP 8							
300 mg/kg/day	males	7188[a]	6577a	0.5	715	411	0.5
(with impurities)							
	females	4662	2032	0.5	747	232	4
GROUP 9							
300 mg/kg/day	males	1294	752	0.5	436	187	0.5
(without impurities)							
	females	6994	3810	0.5	1018	366	4

[a] Includes outlying value for rat 93–6128. Omitting the outlier yields an AUC of 1081 ng/ml·hr and a C_{max} of 470 ng/ml.

2) Plasma levels of parent (and metabolite on day 10) were several fold higher in F than in M.

3) AUCs of parent compound were several-fold greater on day 82 than on day 10 in males. (A smaller increase was seen in F at the higher doses). There was no clear difference in AUC of the metabolite between days 10 and 82.

4) On day 10, levels of parent and metabolite were generally similar to each other (except for greater levels of parent in LD and MD F). On day 82, levels of parent were generally several fold greater than those of the metabolite at all doses in both sexes.

5) On day 10 in both sexes and on day 82 in M, levels of parent and metabolite at HD were equal to or somewhat greater than those at HD*; the reverse was true in F on day 82. (i.e. levels somewhat greater in HD*).

It should be noted that the above conclusions are far from definitive in view of the small N and large inter-animal variation noted.

SEGMENT II REPRODUCTION IN RATS:

A) Dosage

30 F at 0, 75 (LD), 150 (MD), or 300 (HD) mg/kg/day of "degraded" drug (i.e., containing impurities), or 300 mg/kg/day of undegraded drug (this group hereafter referred to as HD*), days 6-15 of gestation, by gavage.

Dams sacrificed day 20 of gestation. Live fetuses examined externally; 1/2 examined for visceral effects using a fresh tissue dissection method (+ Wilson sectioning of head); these + remaining fetuses examined for skeletal effects with alcian blue/alizarin red S.

Strain: : CD BR Charles River

B) Results

1) Observed signs

a) Hyperactivity at all doses, D-R; similar incidence at HD and HD*; not seen after day 9 of gestation.

b) "Rooting in bedding" (said to be consistent with "taste aversion") seen at all doses, generally D-R; similar incidence at HD and HD*.

2) Mortality

None

3) Bodyweight

Decreased gain/slight loss at all doses, D-R, days 6-9 of gestation; similar effect at HD and HD*. At the end of the dosing period (day 16 of gestation) weight was 97%, 97%, 94%, and 93% of control at LD, MD, HD, and HD*, resp. Treated groups tended to gain more than controls day 18-20 of gestation although on day 20 weights were still slightly below control.

4) Food Consumption

Decreased at all doses, D-R, esp. during days 6-9 of gestation. No differences between HD and HD*. Consumption tended to be slightly increased above controls days 16-20 of gestation.

5) Reproductive data

Pregnancy rate was slightly decreased at HD and HD* (approx. 85% vs 97% in control).

No drug effects on the various parameters (see attached table), including pre- and post-implantation loss, sex ratio, fetal weight and length, etc.

6) Fetal exam

No drug effects (See attached tables).

7) PK data

(This was actually a separate study using 16/sex/dose. On day 15 of gestation, plasma was sampled pre-dosing and at 0.5, 1, and 4 hr. post-dosing [4/dose/time point] and assayed for bupropion and 3 of its metabolites [306U73, 494U72, and 17U67]).

Results for bupropion and 306U73 are shown in the attached table. (The other 2 metabolites were not detected in any sample). Plasma levels of parent compound were similar at all doses; levels of the metabolite were roughly dose-proportional. There were no differences in PK parameters between HD and HD*. (Note the small N and large inter-animal variation makes any conclusion problematic). (Also note that in comparison to levels obtained in F in the 90 day toxicity study, above, levels of bupropion were roughly similar, but levels of 306U73 were much higher in the present study).

Table 5. Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex, Body Weights and Crown Rump Length

	323U66 HCl (mg/kg/day, po)				
	0	75	150	300	300[a]
ALL LITTERS[b]	29	27	27	25	24
No. Corpora Lutea per Dam[c]	16.97 ±0.46 N=29	15.89 ±0.40 N=27	17.19 ±0.41 N=27	16.68 ±0.48 N=25	15.71 ±0.43 N=24
No. Implantation Sites per Litter[c]	15.86 ±0.54 N=29	14.74 ±0.72 N=27	16.37 ±0.30 N=27	16.00 ±0.57 N=25	14.71 ±0.64 N=24
Percent Preimplantation Loss per Litter[c]	7.34 ±2.69 N=29	9.12 ±3.60 N=27	4.52 ±1.17 N=27	6.56 ±2.43 N=25	7.83 ±3.17 N=24
No. Resorptions per Litter[c]	0.59 ±0.16 N=29	0.52 ±0.15 N=27	0.81 ±0.15 N=27	0.88 ±0.25 N=25	0.71 ±0.19 N=24
Percent Resorptions per Litter[c]	3.56 ±0.97 N=29	5.17 ±1.99 N=27	4.89 ±0.90 N=27	5.57 ±1.60 N=25	4.60 ±1.20 N=24
No. Litters with Resorptions	11	10	16	14	11
% Litters with Resorptions	37.93	37.04	59.26	56.00	45.83

(continued)

Table 5. Summary and Statistical Analysis of Uterine Contents (continued)

	323U66 HCl (mg/kg/day, po)				
	0	75	150	300	300[a]
No. Late Fetal Deaths per Litter[c]					
	0.00 ±0.00 N=29	0.00 ±0.00 N=27	0.04 ±0.04 N=27	0.08 ±0.06 N=25	0.00 ±0.00 N=24
Percent Late Fetal Deaths per Litter[c]					
	0.00 ±0.00 N=29	0.00 ±0.00 N=27	0.21 ±0.21 N=27	0.50 ±0.35 N=25	0.00 ±0.00 N=24
No. Litters with Late Fetal Deaths					
	0	0	1	2	0
% Litters with Late Fetal Deaths					
	0.00	0.00	3.70	8.00	0.00
No. Nonlive Implants per Litter[c,d]					
	0.59 ±0.16 N=29	0.52 ±0.15 N=27	0.85 ±0.17 N=27	0.96 ±0.29 N=25	0.71 ±0.19 N=24
Percent Nonlive Implants per Litter[c,d]					
	3.56 ±0.97 N=29	5.17 ±1.99 N=27	5.09 ±0.98 N=27	6.07 ±1.80 N=25	4.60 ±1.20 N=24
No. Litters with Nonlive Implants[d]					
	11	10	16	15	11
% Litters with Nonlive Implants[d]					
	37.93	37.04	59.26	60.00	45.83

(continued)

Table 5. Summary and Statistical Analysis of Uterine Contents (continued)

	323U66 HCl (mg/kg/day, po)				
	0	75	150	300	300[a]
No. Adversely Affected Implants per Litter[c,e]					
	2.24 ±0.52 N=29	1.85 ±0.44 N=27	2.15 ±0.46 N=27	2.08 ±0.45 N=25	2.58 ±0.51 N=24
Percent Adversely Affected Implants per Litter[c,e]					
	13.67 ±3.15 N=29	13.88 ±3.46 N=27	13.02 ±2.76 N=27	13.51 ±3.04 N=25	17.92 ±3.29 N=24
No. Litters with Adversely Affected Implants[e]					
	18	16	20	19	18
% Litters with Adversely Affected Implants[e]					
	62.07	59.26	74.07	76.00	75.00
LIVE LITTERS[f]	29	27	27	25	24
No. Live Fetuses per Litter[c]					
	15.28 ±0.53 N=29	14.22 ±0.78 N=27	15.52 ±0.28 N=27	15.04 ±0.62 N=25	14.00 ±0.63 N=24
Percent Male Fetuses per Litter[c]					
	57.06 ‡ ±2.68 N=29	51.44 ±3.23 N=27	49.31 ±2.76 N=27	47.74 ±2.54 N=25	54.98 ±2.66 N=24
No. Male Fetuses per Litter[c]					
	8.72 ±0.51 N=29	6.96 ±0.48 N=27	7.83 ±0.43 N=27	7.12 ±0.45 N=25	7.71 ±0.52 N=24
No. Female Fetuses per Litter[c]					
	6.55 ±0.47 N=29	7.26 ±0.61 N=27	7.89 ±0.46 N=27	7.92 ±0.50 N=25	6.29 ±0.46 N=24

(continued)

Table 5. Summary and Statistical Analysis of Uterine Contents (continued)

	323U66 HCl (mg/kg/day, po)				
	0	75	150	300	300[a]
Average Fetal Body Weight (g) per Litter[c]					
#	3.642 ±0.051 N=29	3.890 ±0.167 N=27	3.643 ±0.051 N=27	3.616 ±0.045 N=25	3.686 ±0.054 N=24
Average Male Fetal Body Weight (g) per Litter[c]					
#	3.723 ±0.053 N=29	3.985 ±0.164 N=27	3.744 ±0.054 N=27	3.703 ±0.046 N=25	3.748 ±0.059 N=24
Average Female Fetal Body Weight (g) per Litter[c]					
	3.541 ±0.052 N=29	3.630 ±0.048 N=27	3.649 ±0.052 N=27	3.543 ±0.042 N=25	3.608 ±0.057 N=24
Repeated Measures: Bartlett's ($p<0.0001$); DOSE ($p=0.0566$); SEX ($p\leq0.0001$); DOSExSEX ($p=0.4502$).					
Average Fetal Crown Rump Length (mm) per Litter[c]					
	35.5 ± 0.3 N=29	36.3 ± 0.4 N=27	35.7 ± 0.2 N=27	35.5 ± 0.2 N=25	35.6 ± 0.2 N=24
Average Male Fetal Crown Rump Length (mm) per Litter[c]					
	35.8 ± 0.3 N=29	36.6 ± 0.4 N=27	36.1 ± 0.2 N=27	35.8 ± 0.2 N=25	35.8 ± 0.2 N=24
Average Female Fetal Crown Rump Length (mm) per Litter[c]					
	35.1 ± 0.3 N=29	35.5 ± 0.2 N=27	35.2 ± 0.3 N=27	35.2 ± 0.3 N=25	35.2 ± 0.2 N=24
Repeated Measures: Bartlett's ($p=0.0113$); DOSE ($p=0.2189$); SEX ($p\leq0.0001$); DOSExSEX ($p=0.7016$).					

(continued)

Table 5. Summary and Statistical Analysis of Uterine Contents (concluded)

[a]300 mg/kg/day group without impurities as presently marketed.
[b]Includes all dams pregnant at sacrifice; litter size = no. implantation sites per dam.
[c]Reported as the mean ± S.E.M.
[d]Nonlive = late fetal deaths plus resorption.
[e]Adversely affected = nonlive plus malformed.
[f]Includes only dams with live fetuses; litter size = no. live fetuses per dam.
[#]Bartlett's test for homogeneity of variances was either significant ($p<0.0010$) or there was zero variance in one or more groups so the test could not be done, therefore nonparametric statistical tests were employed for evaluation of the data.
[‡]$p<0.05$; ANOVA.

Table 7. Morphological Defects Observed in CD Rat Fetuses: Listing by Defect Type[a]

	323U86 HCl (mg/kg/day,po)				
	0	75	150	300	300[b]
ANY MALFORMATIONS					
Total No. of Fetuses Examined for Any Malformations[c]	443	384	419	376	336
No. of Fetuses with Any Malformations[d]	48	36	35	28	45
% Fetuses with Any Malformations	10.8%	9.4%	8.4%	7.4%	13.4%
Total No. of Litters Examined for Any Malformations[e]	29	27	27	25	24
No. of Litters with Any Malformations[f]	12	11	11	10	14
% Litters with Any Malformations	41.4%	40.7%	40.7%	40.0%	58.3%
EXTERNAL MALFORMATIONS					
Total No. of Fetuses Examined for External Malformations[c]	443	384	419	376	336
No. of Fetuses with External Malformations[d]	0	0	0	0	0
% Fetuses with External Malformations	0.0%	0.0%	0.0%	0.0%	0.0%
Total No. of Litters Examined for External Malformations[e]	29	27	27	25	24
No. of Litters with External Malformations[f]	0	0	0	0	0
% Litters with External Malformations	0.0%	0.0%	0.0%	0.0%	0.0%
VISCERAL MALFORMATIONS					
Total No. of Fetuses Examined for Visceral Malformations[c]	222	192	209	190	168
No. of Fetuses with Visceral Malformations[d]	48	36	35	28	44
% Fetuses with Visceral Malformations	21.6%	18.8%	16.7%	14.7%	26.2%
Total No. of Litters Examined for Visceral Malformations[e]	29	27	27	25	24
No. of Litters with Visceral Malformations[f]	12	11	11	10	13
% Litters with Visceral Malformations	41.4%	40.7%	40.7%	40.0%	54.2%
Hydrocephaly: Mild		4(1)		2(2)	
Hydronephrosis: Right				1(1)	
Hydroureter: Bilateral	26(9)	20(8)	19(8)	17(8)	22(8)
Left	22(11)	16(9)	16(10)	8(5)	22(12)
Right				1(1)	

(continued)

Table 7. Morphological Defects Observed in CD Rat Fetuses (continued)

	323U66 HCl (mg/kg/day,po)				
	0	75	150	300	300[b]
SKELETAL MALFORMATIONS					
Total No. of Fetuses Examined for Skeletal Malformations[c]	443	384	419	376	336
No. of Fetuses with Skeletal Malformations[d]	0	0	0	0	1
% Fetuses with Skeletal Malformations	0.0%	0.0%	0.0%	0.0%	0.3%
Total No. of Litters Examined for Skeletal Malformations[e]	29	27	27	25	24
No. of Litters with Skeletal Malformations[f]	0	0	0	0	1
% Litters with Skeletal Malformations	0.0%	0.0%	0.0%	0.0%	4.2%
Bipartite Cartilage, Dumbbell Ossification Center:					
Thoracic Centrum					1(1)
ANY VARIATIONS					
Total No. of Fetuses Examined for Any Variations[c]	443	384	419	376	336
No. of Fetuses with Any Variations[d]	202	178	197	178	158
% Fetuses with Any Variations	45.6%	46.4%	47.0%	47.3%	47.0%
Total No. of Litters Examined for Any Variations[e]	29	27	27	25	24
No. of Litters with Any Variations[f]	29	27	27	25	24
% Litters with Any Variations	100.0%	100.0%	100.0%	100.0%	100.0%
EXTERNAL VARIATIONS					
Total No. of Fetuses Examined for External Variations[c]	443	384	419	376	336
No. of Fetuses with External Variations[d]	0	0	0	2	0
% Fetuses with External Variations	0.0%	0.0%	0.0%	0.5%	0.0%
Total No. of Litters Examined for External Variations[e]	29	27	27	25	24
No. of Litters with External Variations[f]	0	0	0	2	0
% Litters with External Variations	0.0%	0.0%	0.0%	8.0%	0.0%
Hematoma: Neck				2(2)	

(continued)

Table 7. Morphological Defects Observed in CD Rat Fetuses (continued)

	323U66 HCl (mg/kg/day,po)				
	0	75	150	300	300[b]
VISCERAL VARIATIONS					
Total No. of Fetuses Examined for Visceral Variations[c]	222	192	209	190	166
No. of Fetuses with Visceral Variations[d]	194	172	191	173	152
% Fetuses with Visceral Variations	87.4%	89.6%	91.4%	91.1%	90.5%
Total No. of Litters Examined for Visceral Variations[e]	29	27	27	25	24
No. of Litters with Visceral Variations[f]	29	27	27	25	24
% Litters with Visceral Variations	100.0%	100.0%	100.0%	100.0%	100.0%
Enlarged Lateral Ventricle (Full): Bilateral	44(16)	34(17)	34(16)	48(18)	33(17)
Left	9(7)	7(4)	6(5)	6(3)	4(4)
Right	6(4)	7(5)	8(7)		8(7)
Enlarged Lateral Ventricle (Half): Bilateral	39(19)	39(19)	49(21)	39(16)	28(14)
Left	2(2)	2(2)	4(4)		3(3)
Right	7(6)	8(5)	3(3)	9(6)	5(5)
Enlarged Lateral Ventricle (Partial): Bilateral	85(24)	76(25)	84(23)	76(23)	68(23)
Left	5(4)	9(7)	6(6)	1(1)	5(4)
Right	4(4)		6(6)	1(1)	5(5)
Distended Ureter: Bilateral		1(1)	2(2)	1(1)	1(1)
Left	3(3)	3(3)	1(1)	2(2)	2(2)
Right		2(2)	2(2)	1(1)	1(1)
SKELETAL VARIATIONS					
Total No. of Fetuses Examined for Skeletal Variations[c]	443	384	419	376	336
No. of Fetuses with Skeletal Variations[d]	17	10	9	9	10
% Fetuses with Skeletal Variations	3.8%	2.6%	2.1%	2.4%	3.0%
Total No. of Litters Examined for Skeletal Variations[e]	29	27	27	25	24
No. of Litters with Skeletal Variations[f]	12	7	5	8	5
% Litters with Skeletal Variations	41.4%	25.9%	18.5%	32.0%	20.8%
Rib on Lumbar I: Bilateral Full			1(1)		
Bilateral Rudimentary	2(2)		1(1)	2(1)	1(1)
Left Full	1(1)				
Left Rudimentary	4(3)	3(3)		1(1)	2(2)
Right Rudimentary	4(3)	3(2)	1(1)	1(1)	1(1)
Short Rib: XIII	1(1)				
Wavy Rib				1(1)	1(1)
Normal Cartilage, Bipartite Ossification Center:					
Thoracic Centrum	2(2)	4(4)	7(4)	3(3)	5(3)
Dumbbell Cartilage, Normal Ossification Center:					
Thoracic Centrum	1(1)				
Dumbbell Cartilage, Dumbbell Ossification Center:					
Thoracic Centrum	3(3)			1(1)	

(continued)

Table 7. Morphological Defects Observed in CD Rat Fetuses (concluded)

[a]A single fetus may be represented more than once in listing individual defects. Data indicate number of fetuses (number of litters).
[b]300 mg/kg/day group without impurities as presently marketed.
[c]Only live fetuses were examined.
[d]Fetuses with one or more malformations/variations.
[e]Includes only litters with live fetuses.
[f]Litters with one or more malformed/variant fetuses.

9J

Table 5. Pharmacokinetic Parameters for Bupropion and 306U73 in Pregnant Rats on Day 15 of Gestation after an Oral Administration of Bupropion (TOX 652)

	Bupropion			306U73		
	AUC (ng/mL·hr)	C_{max} (ng/mL)	T_{max} (hr)	AUC (ng/mL·hr)	C_{max} (ng/mL)	T_{max} (hr)
GROUP 1 75 mg/kg/day (with impurities)	3119	1848	0.5	1912	570	0.5
GROUP 2 150 mg/kg/day (with impurities)	2162	2056	0.5	2682	945	0.5
GROUP 3 300 mg/kg/day (with impurities)	2885	1766	0.5	6899	1916	0.5
GROUP 4 300 mg/kg/day (without impurities)	3085	1666	0.5	6696	2182	0.5

GENOTOXICITY STUDIES

A) Ames Tests

1) Plate incorporation method

Results summarized in attached tables. (Data for TA 1538 generated in a separate experiment because in original experiment vehicle control values were outside normal range).

Bupropion was considered positive in strain TA 100, in both presence and absence of metabolic activation. The maximum number of revertants was about 2 x control (which was the criterion for a positive response in this strain). An increase of about 2.5 x was seen in TA 1535 in the presence of metabolic activation; however the criterion for a positive response in this strain was a 3 x increase.

2) Preincubation Method

Results summarized in attached table. As indicated, the pattern of response was the same as in the plate incorporation assay (i.e., increase in TA 100 with and without activation and in TA 1535 with activation); however in no case was the stated criterion for a positive response achieved (i.e., 2 x increase for TA 100 and 3 x for TA 1535).

B) In vivo cytogenetic study in rats

1) Dosage

5/sex at 0, 0, 100 (LD), 200 (MD), or 300 (HD) mg/kg/day of "degraded" drug (i.e., containing impurities), or 300 mg/kg/day of undegraded drug (this group hereafter referred to as HD*), for 5 consecutive days; animals sacrificed 24 hr. after last dose for cytogenetic exam of bone marrow.

Strain: Charles River CD

2) Results

a) Observed signs

Salivation at all doses, D-R; no difference in incidence between HD and HD*.

b) Mortality

None

TABLE 2
MUTAGENICITY ASSAY RESULTS
SUMMARY
PLATE INCORPORATION

TEST ARTICLE ID: 323U66 HCl

EXPERIMENT ID: 15897-B1

DATE PLATED: 18-Oct-93

DATE COUNTED: 28-Oct-93

	DOSE/PLATE	MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACKGROUND LAWN*
		TA98		TA100		TA1535		TA1537		TA1538		
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: Rat Liver												
VEHICLE CONTROL[1]		31	2	121	14	11	3	7	2	998	3	1
VEHICLE CONTROL[2]		26	2	121	3	13	5	6	2	900	17	1
TEST ARTICLE	60.0 µg	27	3	150	7	20	7	9	3	BC	-	1
	300 µg	32	4	240	19	26	11	8	4	BC	-	1
	1500 µg	32	4	228	22	20	3	8	4	BC	-	1
	3000 µg	28	4	196	28	23	8	7	1	BC	-	1
	6000 µg	14	5	12	14	12	4	1	1	BC	-	1
POSITIVE CONTROL **		903	70	1065	11	199	25	156	19	1319	55	1
MICROSOMES: None												
VEHICLE CONTROL[1]		21	5	99	7	10	2	6	2	720	2	1
VEHICLE CONTROL[2]		19	5	102	15	13	3	5	3	700	10	1
TEST ARTICLE	60.0 µg	17	3	120	12	9	4	5	1	BC	-	1
	300 µg	11	3	135	22	11	7	7	1	BC	-	1
	1500 µg	18	6	163	8	11	4	5	1	BC	-	1
	3000 µg	22	3	200	21	18	3	5	0	BC	-	1
	6000 µg	0	0	0	0	0	0	0	0	BC	-	5
POSITIVE CONTROL ***		182	20	331	35	394	20	219	37	322	17	1

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
TA1538 2-aminoanthracene 2.5 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
TA1538 2-nitrofluorene 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal — 2 = slightly reduced — 3 = moderately reduced
4 = extremely reduced — 5 = absent — 6 = obscured by precipitate
sp = slight precipitate — mp = moderate precipitate (requires hand count) — hp = heavy precipitate (requires hand count)

[1] Deionized water vehicle control (BMA Batch 272), 200 µl.
[2] 0.001 N HCl vehicle control (supplied by the Sponsor), 200 µl.

@ The mean vehicle control values for tester strain TA1538 were not within the acceptable range. See retest data, Table 3.

BC = Not counted due to unacceptable mean vehicle control value. See retest data, Table 3.

TABLE 3
MUTAGENICITY ASSAY RESULTS
INDIVIDUAL PLATE COUNTS AND SUMMARY
PLATE INCORPORATION

TEST ARTICLE ID: 323U66 HCl — EXPERIMENT ID: 15897-B2

DATE PLATED: 29-Oct-93 — DATE COUNTED: 01-Nov-93

DOSE/PLATE	REVERTANTS PER PLATE TA1538 1	2	3	MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION TA1538 MEAN	S.D.	BACKGROUND LAWN*
MICROSOMES: Rat Liver						
VEHICLE CONTROL[1]	26	16	16	19	6	1
VEHICLE CONTROL[2]	16	8	20	15	6	1
TEST ARTICLE 60.0 µg	27	17	23	22	5	1
300 µg	24	18	17	20	4	1
1500 µg	12	21	26	20	7	1
3000 µg	21	15	22	19	4	1
6000 µg	16	9	9	11	4	1
POSITIVE CONTROL **	1048	1234	1281	1188	123	1
MICROSOMES: None						
VEHICLE CONTROL[1]	6	6	11	8	3	1
VEHICLE CONTROL[2]	16	7	13	12	5	1
TEST ARTICLE 60.0 µg	7	11	13	10	3	1
300 µg	4	11	12	9	4	1
1500 µg	9	13	6	9	4	1
3000 µg	10	10	15	12	3	1
6000 µg	0	0	0	0	0	5
POSITIVE CONTROL ***	192	237	192	207	26	1

** TA1538 2-aminoanthracene 2.5 µg/plate — *** TA1538 2-nitrofluorene 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal — 2 = slightly reduced — 3 = moderately reduced
4 = extremely reduced — 5 = absent — 6 = obscured by precipitate
sp = slight precipitate — mp = moderate precipitate (requires hand count) — hp = heavy precipitate (requires hand count)

[1] Deionized water vehicle control (EMA Batch 272), 200 µl.
[2] 0.001 N HCl vehicle control (supplied by the Sponsor), 200 µl.

TABLE 2
MUTAGENICITY ASSAY RESULTS
SUMMARY
PREINCUBATION

TEST ARTICLE ID: 323U66 HCl EXPERIMENT ID: 15897-B1

DATE PLATED: 20-Oct-93 DATE COUNTED: 28-Oct-93

DOSE/PLATE		TA98 MEAN	TA98 S.D.	TA100 MEAN	TA100 S.D.	TA1535 MEAN	TA1535 S.D.	TA1537 MEAN	TA1537 S.D.	TA1538 MEAN	TA1538 S.D.	BACKGROUND LAWN*
MICROSOMES: Rat Liver												
VEHICLE CONTROL		28	7	137	22	10	1	11	4	16	2	1
VEHICLE CONTROL		25	4	140	4	13	4	9	4	23	3	1
TEST ARTICLE	30.0 µg	28	7	167	13	19	3	7	3	17	7	1
	150 µg	36	3	212	2	31	2	9	3	16	7	1
	300 µg	34	9	231	13	30	6	7	1	15	3	1
	900 µg	25	3	215	9	26	8	9	2	17	3	1
	1800 µg	29	4	168	9	10	2	6	2	11	4	2
POSITIVE CONTROL **		1062	33	1135	33	127	6	140	12	1157	50	1
MICROSOMES: None												
VEHICLE CONTROL		18	3	102	9	15	3	8	2	9	3	1
VEHICLE CONTROL		22	3	106	2	12	1	6	3	11	1	1
TEST ARTICLE	30.0 µg	15	6	123	7	12	4	6	2	10	3	1
	150 µg	17	4	123	15	15	5	8	2	13	4	1
	300 µg	15	8	138	12	13	3	9	2	11	3	1
	900 µg	15	3	1010	87	20	3	60	1	6	3	3
	1800 µg	170	15	0	0	0	0	0	0	40	3	4
POSITIVE CONTROL ***		213	33	794	13	637	24	1153	22	274	23	1

MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
TA1538 2-aminoanthracene 2.5 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
TA1538 2-nitrofluorene 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

1 Deionized water vehicle control (EMA Batch 272), 100 µl.
2 0.001 N HCl vehicle control (supplied by the Sponsor), 100 µl.
3 One or more plates of the triplicate evaluated as having an absent (5) bacterial background lawn.

c) Bodyweight

No drug effect

d) Cytogenetic data

No drug effects (Summary tables attached).

e) PK data

Plasma levels of bupropion and 3 metabolites were measured in separate groups of animals receiving 300 mg/kg/day (degraded or undegraded) for 5 days; samples taken 0.5 hr. after last dose; N = 5/sex/group. Results are shown in the attached table. Levels of bupropion were generally similar to those of metabolite 306U73. (Metabolites 494U72 and 17U67 were below the limit of quantification). Levels of bupropion and metabolite 306U73 were greater in females than in males. There was no clear differences in levels between animals dosed with degraded vs undegraded drug, but this cannot be concluded confidently in view of the small N and large inter-animal variation.

Table 11. Summary of Structural Aberrations

A Cytogenetic Study in Rats with Five Daily Doses of 323U66-HCl (Bupropion; Containing Impurities)

SUMMARY OF STRUCTURAL ABERRATIONS (MUT 223)

Dose (mg/kg)	% Aberrant Cells			No. Aberrations/per 100 Cells			Mitotic Index (%)		
	Males	Females	Combined	Males	Females	Combined	Males	Females	Combined
0	0.8	0.0	0.4	1.2	0.0	0.6	2.96	2.52	2.74
0	0.0	0.4	0.2	0.0	0.4	0.2	2.80	4.66	3.73
100	0.0	0.0	0.0	0.0	0.0	0.0	3.80	3.28	3.54
200	0.0	0.0	0.0	0.0	0.0	0.0	3.08	2.44	2.76
300	0.4	0.0	0.2	0.8	0.0	0.4	2.18	2.60	2.39
300*	0.4	0.4	0.4	0.4	0.4	0.4	2.18	2.44	2.31
Positive Control (CP: 30 mg/kg)	6.0	7.2**	6.6	18.0	14.4	16.2	1.06	1.92	1.49
Positive Control CP: 40 mg/kg)	26.8**	15.6**	21.2	115.2	52.0	83.6	0.54	0.26	0.40

*Test compound used for this group was the material presently used in clinics.
**p<0.05, each test dose group was compared with the pooled vehicle control males or females for statistical significance (see Appendix 3)

11 a

Table 12. Percentage of Cells with Non–Diploid Chromosome Nos

A Cytogenetic Study in Rats with Five Daily Doses of 323U66-HCl (Bupropion; Containing Impurities)

PERCENTAGE OF CELLS WITH NON-DIPLOID CHROMOSOME NUMBERS (MUT 223)

Dose (mg/kg)	% Cells:	24 Hr. Sacrifice Males	Females	Combined
0	Hypodiploid:	14.8	7.6	11.2
	Hyperdiploid:	0.0	0.0	0.0
	Polyploid:	0.0	0.0	0.0
0	Hypodiploid:	6.8	8.4	7.6
	Hyperdiploid:	0.4	0.0	0.2
	Polyploid:	0.0	0.0	0.0
100	Hypodiploid:	12.8	16.0	14.4
	Hyperdiploid:	0.4	1.2	0.8
	Polyploid:	0.0	0.0	0.0
200	Hypodiploid:	14.4	11.2	12.8
	Hyperdiploid:	0.0	0.0	0.0
	Polyploid:	0.0	0.0	0.0
300	Hypodiploid:	10.8	8.8	9.8
	Hyperdiploid:	0.8	0.4	0.6
	Polyploid:	0.0	0.0	0.0
300*	Hypodiploid:	11.2	6.8	9.0
	Hyperdiploid:	0.0	0.4	0.2
	Polyploid:	0.0	0.0	0.0
Positive Control (CP: 30 mg/kg)	Hypodiploid:	10.4	12.0	11.2
	Hyperdiploid:	0.4	0.4	0.4
	Polyploid:	0.0	0.0	0.0
Positive Control (CP: 40 mg/kg)	Hypodiploid:	16.0	16.8	16.4
	Hyperdiploid:	1.2	0.0	0.6
	Polyploid:	0.0	0.0	0.0

* Test compund used for this group was the material presently used in clinics.

11 c

Table 2. Individual Plasma Concentrations of Bupropion and 306U73 in Rats on Day 5 Following an Oral Administration of Bupropion (MUT 223)

	UAIN[a] 93-Prefix	Gender	Time (hr)	Bupropion (ng/mL)	306U73 (ng/mL)
GROUP 9	6657	male		181	349
300 mg/kg/day	6658	male		125	174
(with impurities)	6659	male		89	81
	6660	male		432	215
	6661	male		483	471
GROUP 10	6662	male		106	225
300 mg/kg/day	6663	male		115	271
(without impurities)	6664	male		92	221
	6665	male		147	337
	6666	male		122	365
GROUP 9	6712	female		595	883
300 mg/kg/day	6713	female		2040	1246
(with impurities)	6714	female		3046	2226
	6715	female		929	1021
	6716	female		430	561
GROUP 10	6717	female		2285	693
300 mg/kg/day	6718	female		391	114
(without impurities)	6719	female		730	1067
	6720	female		1087	555
	6721	female		1247	1164

[a] Unique Animal Identification Number

SUMMARY

Bupropion (B) is currently marketed as an immediate release formulation; the usual animal toxicity studies were performed and reviewed under NDA 18-644 (Original Summary of 6/15/82). The new sustained release formulation contains 2 new degradation products at proposed limits of 2% each, as well as apparently increased amounts of other degradation products/impurities with limits ranging from % each, with a total limit of 8%. A total of 8% represents 32 mg/day at the maximum recommended dose (400 mg) in humans. We thus requested that the sponsor perform bridging studies with drug containing the maximum allowable amounts of impurities in the new formulation, also to include a group receiving drug containing the impurity profile which was present in the original toxicity testing of the currently marketed drug substance. (My memo of 5/24/93, Dr. Fitzgerald's memo of 6/7/93, and letter to sponsor of 6/9/93). The sponsor's summary of the levels of impurities in the various studies performed are as follows. (Note that the impurity levels in the original formulation used as a comparison group in these studies were not given):

	%						
	3 Chloro-benzoic Acid	268W93	3134W92	269W93	852U77	20U78	827U76
14 Day Toxicity Study in Rats	-	-	-	-	10.3	-	-
~~28~~ 90 Day Toxicity Study in Rats	0.7	2.7	0.8	1.8	6.2	2.4	1.3
Ames Mutagenicity Assay	1.1	1.7	0.7	2.1	5.9	2.4	1.7
Cytogenicity Study in Rats	0.7	3.1	0.8	2.1	6.5	2.5	1.2
Teratogenicity Study in Rats	0.7	2.6	0.7	1.4	6.3	2.3	1.2

In the 90 day rat study (75, 150, 300 mg/kg/day, plus 300 mg/kg/day of original formulation, by gavage), drug effects included salivation (all doses), increased liver weights (all doses), minimal centrilobular hepatocellular hypertrophy (seen at HD; lower doses not examined), and increased incidence of very minimal-to-mild chronic progressive nephrosis in HD M (lower doses not examined). Thyroid and adrenal weights were slightly increased in HD M without histological correlates. There were no clear differences between the old and new formulations. (Plasma level data obtained in this study indicated that levels of bupropion, but not metabolite 306U73, were greater on day 82 than on day 10 of treatment. This is in disagreement with previous data suggesting that B can induce its own metabolism, as well as the sponsor's contention that the histological effects of B on the liver [also seen in previous studies] are secondary to enzyme induction. However, it is noted that the plasma level data are not definitive in view of the small N and large inter-animal variation noted. It was also seen that levels of bupropion in F were several fold higher than in M, in agreement with other studies).

(A 14 day rat study was also performed at doses of 100, 200, and 300 mg/kg/day; however, the drug used did not contain many of the impurities of interest and did not use a comparison group dosed with the original formulation. Results were generally compatible with the 90 day study [salivation, increased liver weights] but no histopathological effects were seen, implying that more than 14 days' treatment are needed to show such effects.

B was not teratogenic in a segment II study in Charles River rats (75, 150, 300 mg/kg), despite the production of some maternal toxicity. Lack of teratogenicity was also seen in the rat study (Long-Evans strain) performed under NDA 18-644.

In the Ames Test (plate incorporation method) B was borderline positive in strain TA 100 both in the absence and presence of metabolic activation. A slight increase in revertants was seen in TA 1535 in the presence of activation, but this did not meet the stated criteria for a positive response. When the preincubation method was used the pattern of responses was similar to the above although in no case were the stated criteria for a positive response met. Comparison groups using the "original" B were not used in these studies; however the responses seen here were strikingly similar to those seen in the study performed under NDA 18-644.

B (both "old" and "new") was inactive in an in vivo cytogenetic study in rats at oral doses up to 300 mg/kg/day x 5 days. This is in contrast to a study performed under NDA 18-644 in which 300 but not 100 or 200 mg/kg caused an increase in chromosomal aberrations.

LABELLING:

The following animal data-based portions of the labelling differ from the approved labelling of the original formulation. (Note that in most cases the changes are not specific to the sustained release formulation per se but reflect animal data obtained since the original labelling was approved):

1) Page 2-3 ("Pharmacodynamics and Pharmacological Actions")

a) First paragraph

This paragraph was completely rewritten. Not much was said about the mechanism of action of bupropion (B) in the original labelling. It was active in the Porsolt and tetrabenazine models of depression, but was a relatively weak blocker of the neuronal uptake of serotonin, norepinephrine and dopamine. (See my review of NDA 18-644). The sponsor now wishes to state that "the antidepressant activity of bupropion is presumed to be mediated primarily through pathways subserved by norepinephrine as evidenced by its ability to reduce firing rates of norepinephrine-containing neurons in the locus coeruleus at doses which are active in animal antidepressant models". It is not clear if this study has been submitted; the sponsor's reference for this paragraph states "Update of Clinical Pharmacology Section to include information regarding bupropion's effects on the firing rates of noradrenergic neurons in the locus coeruleus". There is a brief summary of relevant data in the sponsor's submission of 1/28/93 to NDA 18-644; according to this, in studies in anesthetized rats, B, metabolite 306U73, and imipramine reduced NE firing rate with i.v. ID_{50s} of 2.7, 1.4, and 0.45 mg/kg, resp. (and an i.p. ID_{50} of 12.6 mg/kg for B). It was also stated that B inhibited the firing rate of A9 and A10 dopamine neurons with i.p. ID_{50s} of 44 and 43 mg/kg, resp; imipramine at 16 mg/kg was said to be without effect. (It was also stated that B, but not its metabolites 306U73 and 494U73 decreased dopaminergic firing rates when given i.v., but doses used were not stated). It was further stated that B did not alter firing rates of dorsal raphe serotonergic neurons up to a cumulative dose of 12.5 mg/kg i.v. or at a dose of 25 mg/kg i.p. (ID_{50} for imipramine = 16 mg/kg i.p.).

Several antidepressants are known to decrease the firing rate of NE and /or 5HT neurons; this is thought to represent a compensatory response to increased synaptic levels of the respective neurotransmitter, in turn caused by blockade of neurotransmitter re-uptake. The mechanism of the decreased firing in the case of B is not clear in that it is a relatively weak reuptake blocker (and somewhat more potent vs DA than NE). (Furthermore the decreased firing rate is not a sufficient condition for the clinical antidepressant effect of antidepressants in that the former occurs after acute dosing whereas the latter occurs only after repeated dosing.) However, even granting that the decrease in firing rate may be relevant to the mechanism of antidepressant action of B, I do not think there are

enough data to say that the antidepressant action of B is mediated primarily through noradrenergic pathways. Although it was said that B was 3-4 x less potent in inhibiting DA (vs NE) firing rate, it is not clear if a time vs response analysis was done, i.e., the relative potency may have been different at different times after dosing. Furthermore, it was previously shown that the effectiveness of B in the Porsolt test was dependent on dopaminergic, but not noradrenergic, neurons (My original review of NDA 18-644). Also, although a relatively weak blocker of neurotransmitter re-uptake, B is more potent against DA uptake than against NE uptake both in vitro and in vivo (although a table in the submission of 1/28/93 to NDA 18-644 indicates that metabolites 306U73 and 494U73 are somewhat more potent against NE [vs DA] in vitro). Also, according to the latter submission there is a study showing that B at a relatively low dose (10 mg/kg i.p.) increased extracellular DA concentration in rat nucleus accumbens. (NE apparently not studied).

Thus, while some of the above results indicate an effect on noradrenergic and/or dopaminergic activity (also, efficacy in the tetrabenazine test per se indicates such activity, as well as the finding of beta-receptor down regulation [although not all studies have found the latter], it is premature to assert the primacy of noradrenergic activity. An appropriate statement in the labelling might be (assuming the data on decreased firing rate has or will be submitted and is adequate):

"The neurochemical mechanism of the antidepressant effect of bupropion is not known. Bupropion is a relatively weak blocker of the neuronal uptake of norepinephrine, serotonin, and dopamine, and does not inhibit monoamine oxidase; however there is some evidence to suggest that its antidepressant action is mediated by noradrenergic and/or dopaminergic mechanisms."

b) Second and third paragraphs

The sponsor wishes to add statements that B administered to mice in a sustained release formulation produced less of an increase in motor activity and fewer convulsions than when administered in an immediate release formulation. The data supporting this are shown in attached tables. Although these data are relatively minimal (e.g., only a single dose was used for the locomotor activity study), these statements are acceptable. (However, the term "significantly" should be removed from both statements; statistical significance is implied by the declaration of an effect). (One might expect that the reason for these pharmacological differences was due to differences in PK between formulations; however as shown in the attached figures there appeared to be little difference in plasma levels of B and metabolites between the 2 formulations.).

Table 2. Comparison of the Seizures Produced in Mice by Oral Doses of a 2 hr Controlled Release Formulation of Bupropion to Those Produced by Oral Doses of Bupropion HCl in Water

Treatment	mg/kg po	Seizure #Conv #dosed	%	Avg. Time to Seizure (min) + SE	Lethality #dead #dosed	%
Bupropion Controlled Release[a]	170.9	0/18	0	—	0/18	0
	366.0	4/20[b]	20	13.7 ± 0.9[c]	1/20	5
Bupropion HCl in water	152	0/12	0	—		0
	304	9/12	75	10.6 ± 2	1/12	8.5
	366	12/12[b]	100	6 ± 1[c]	8/12	66

[a] Calculations of dosage were based on each bead weighing 0.38 mg and containing 0.69 mg bupropion HCl per mg of bead material.

[b] $P < 001$ – Comparing frequency of mice showing seizures after bupropion HCl in water to frequency of mice showing seizures after the same dose formulated in controlled release beads, X^2 Statistic

[c] $P < 0.002$ – Bupropion controlled release vs bupropion HCl in water, students t–test

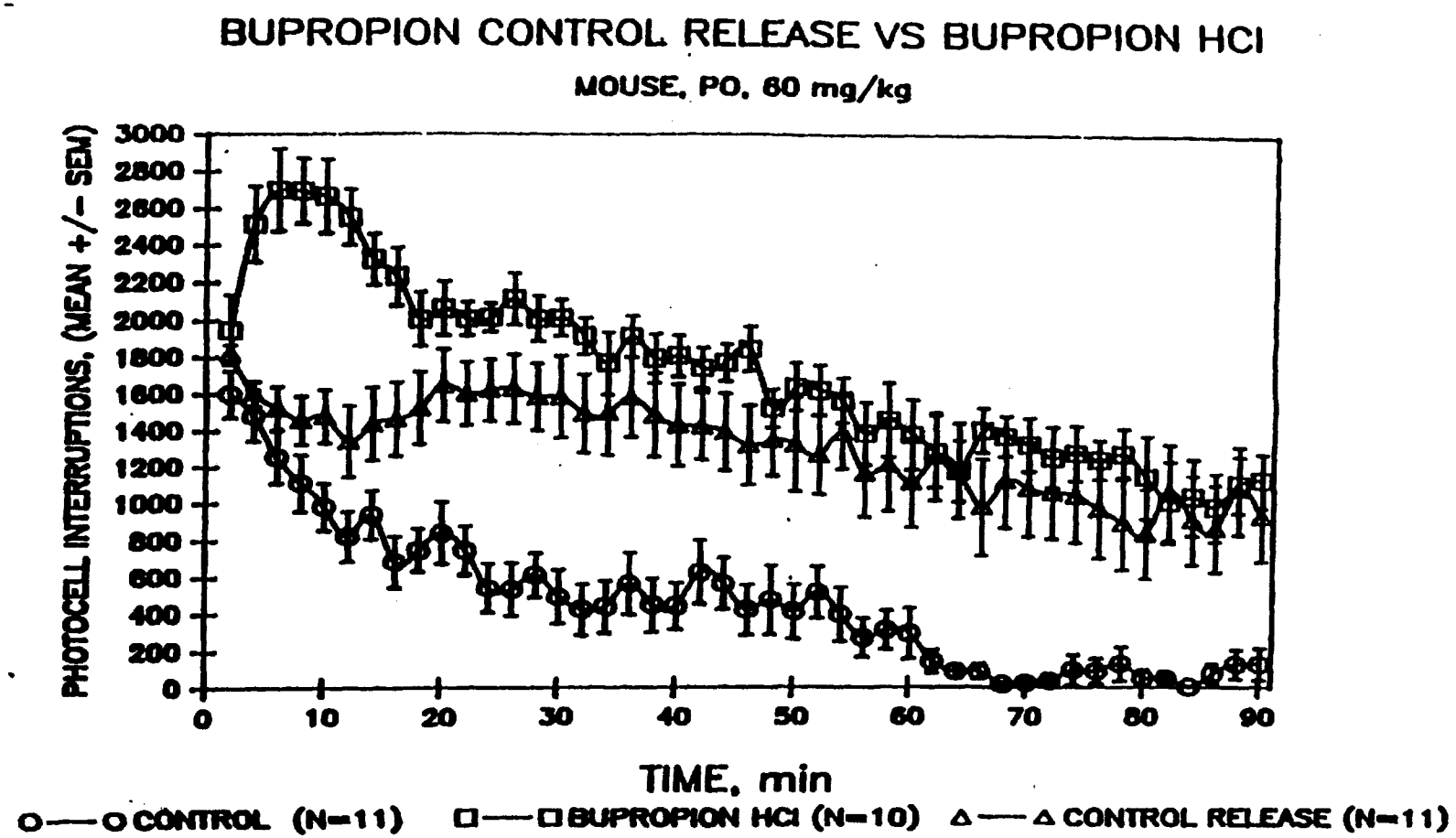


Figure 1.

Effects of oral treatment with either bupropion HCl in water or bupropion formulated in a controlled release matrix on spontaneous locomotor activity of mice in an open field. Values represent photocell beam interruptions that are accumulated over successive 2 minute intervals by an IBM personal computer as described in methods. A single mouse was placed in each cage for testing and means ± S.E.M. are given for the number of mice in parenthesis at the base of the figure.

(The 2 formulations were statistically significantly different from each other only at 0-30 min. interval)

Figure 1

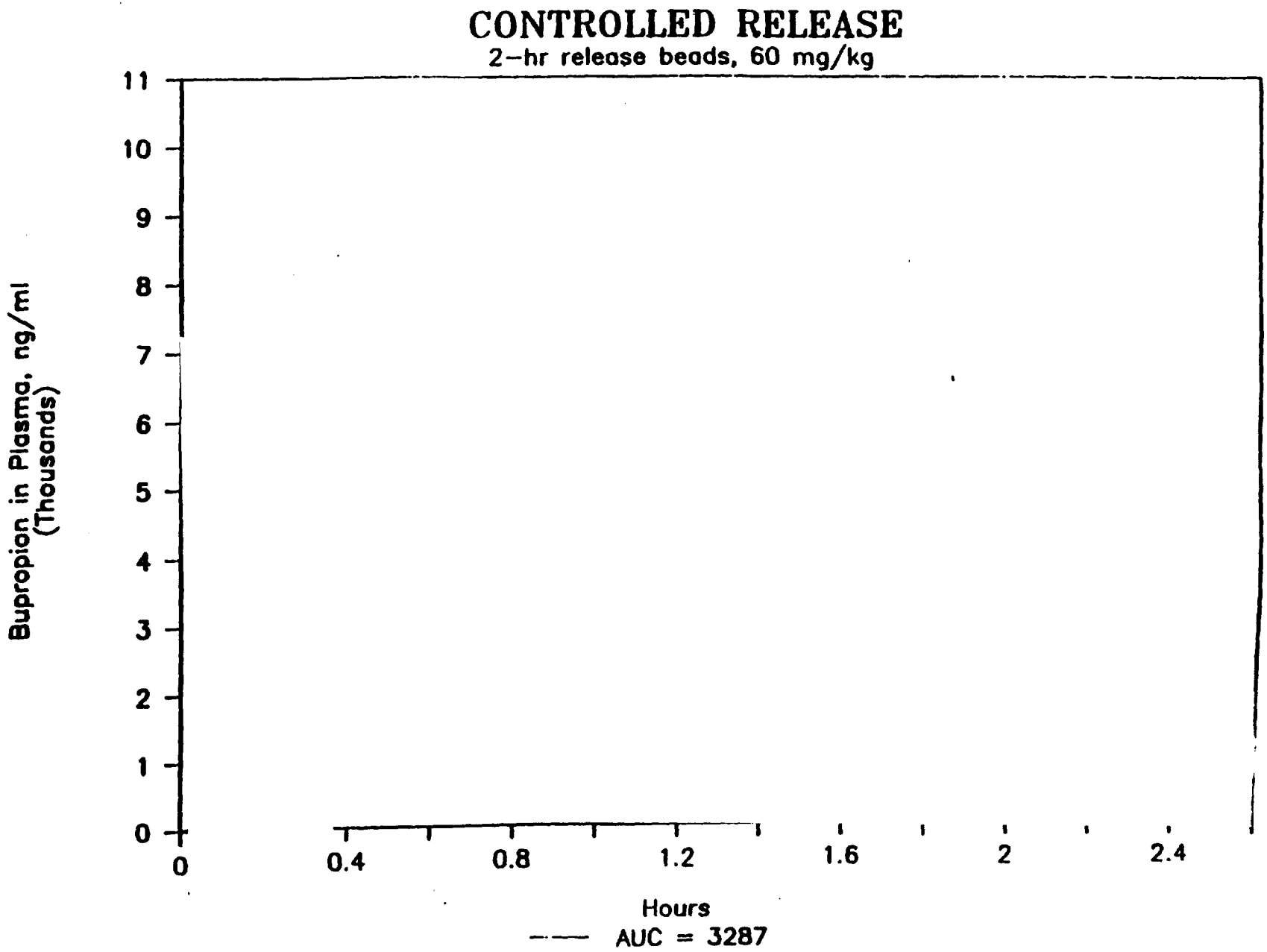

CONTROLLED RELEASE
2–hr release beads, 60 mg/kg
Bupropion in Plasma, ng/ml (Thousands)
11
10
9
8
7
6
5
4
3
2
1
0
0
0.4
0.8
1.2
1.6
2
2.4
Hours
AUC = 3287

Figure 2
INSTANT RELEASE
Dist. Water Soln., 60 mg/kg

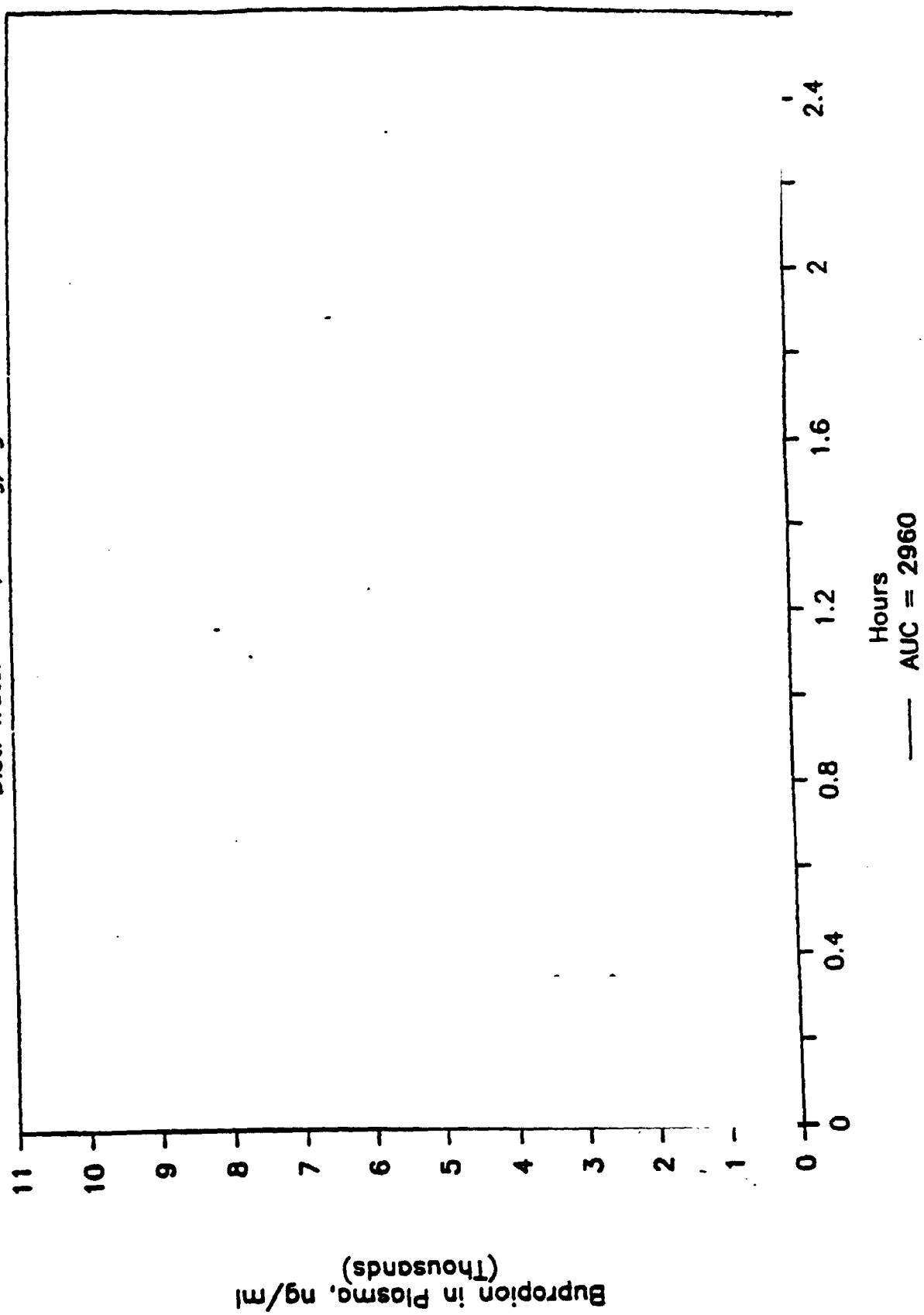


Figure 3

CONTROLLED RELEASE

2-hr Release Beads, 60 mg/kg

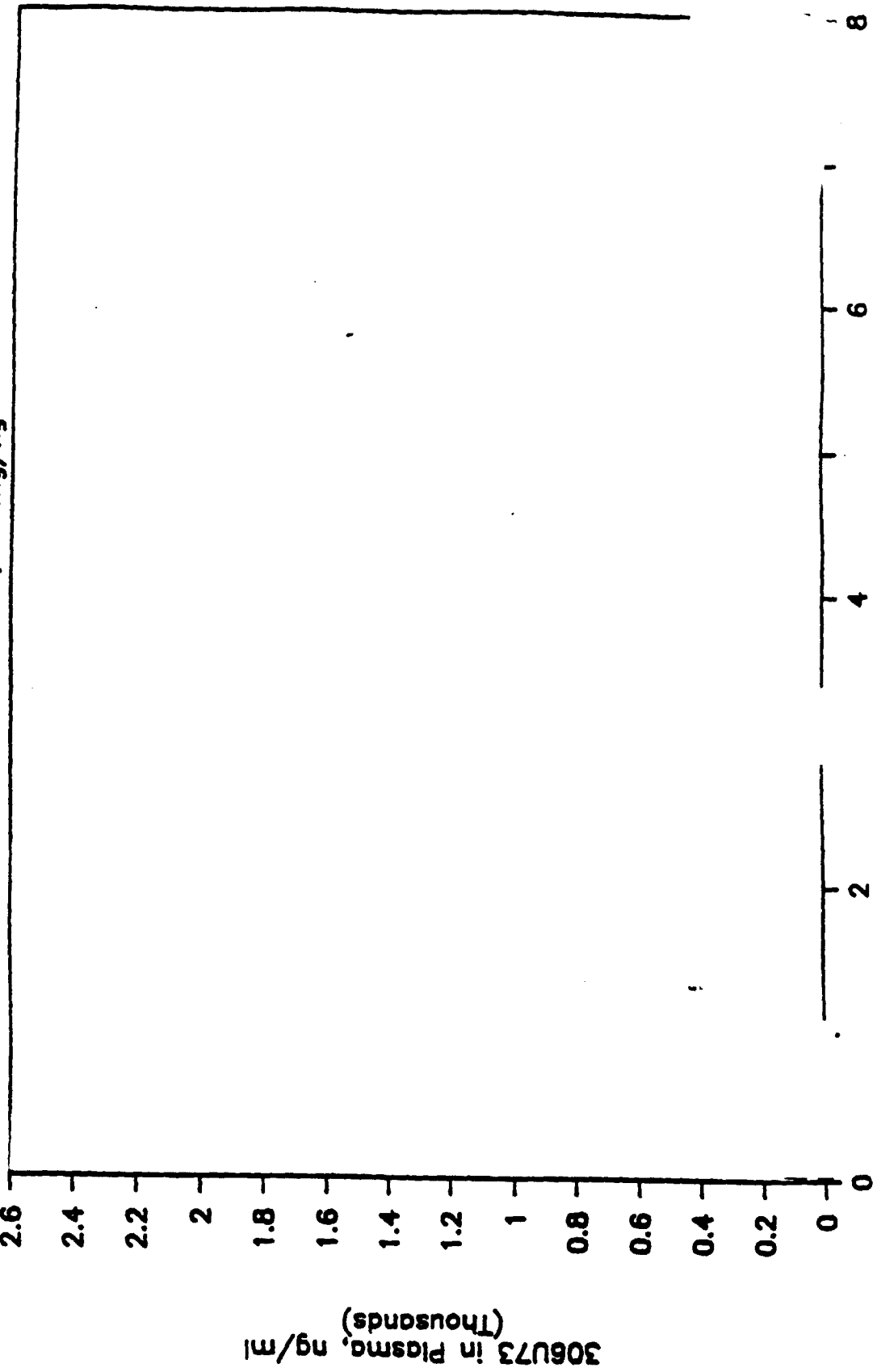


Figure 4

INSTANT RELEASE

Dist. Water Soln., 60 mg/kg

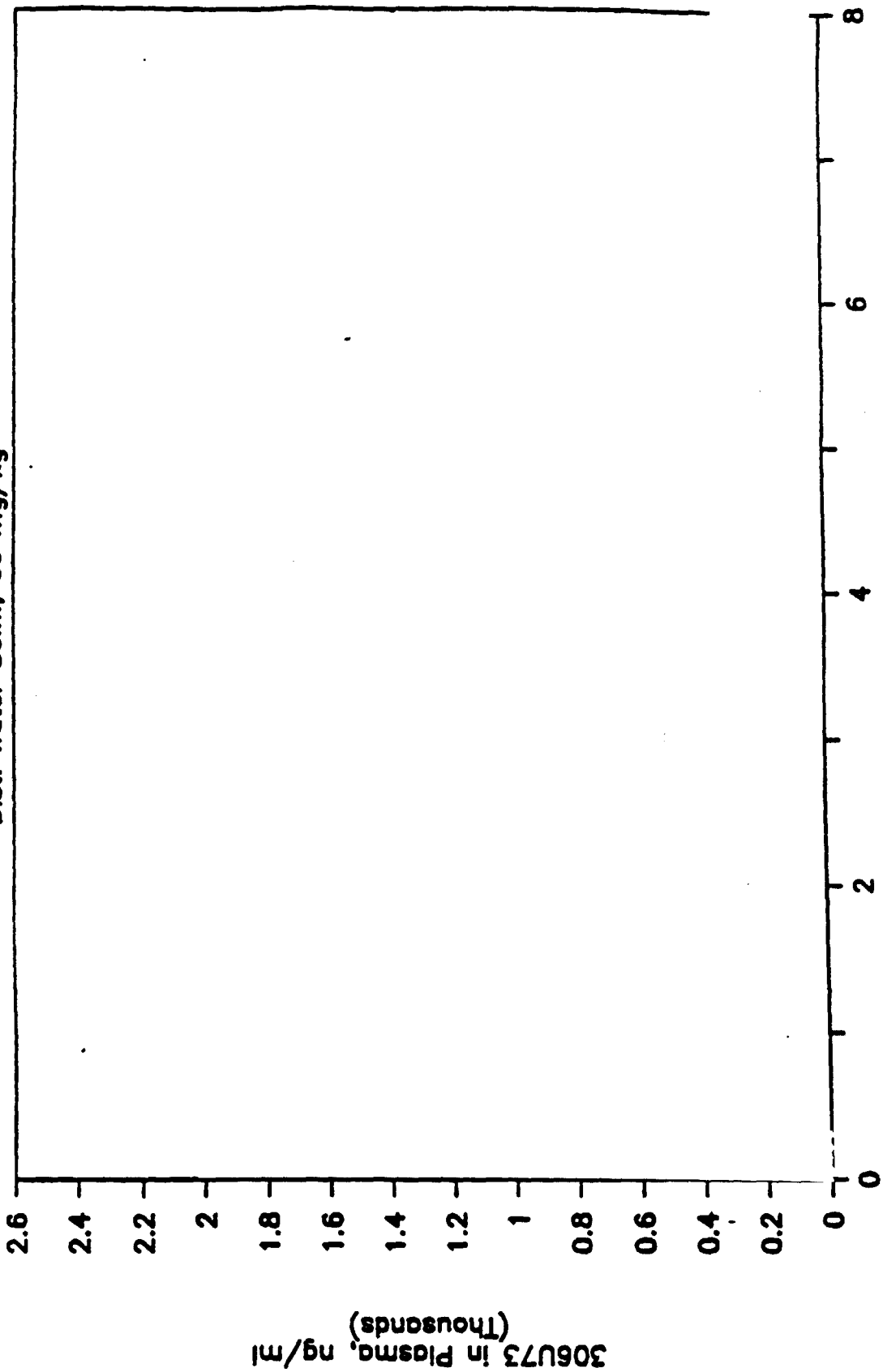


Figure 5

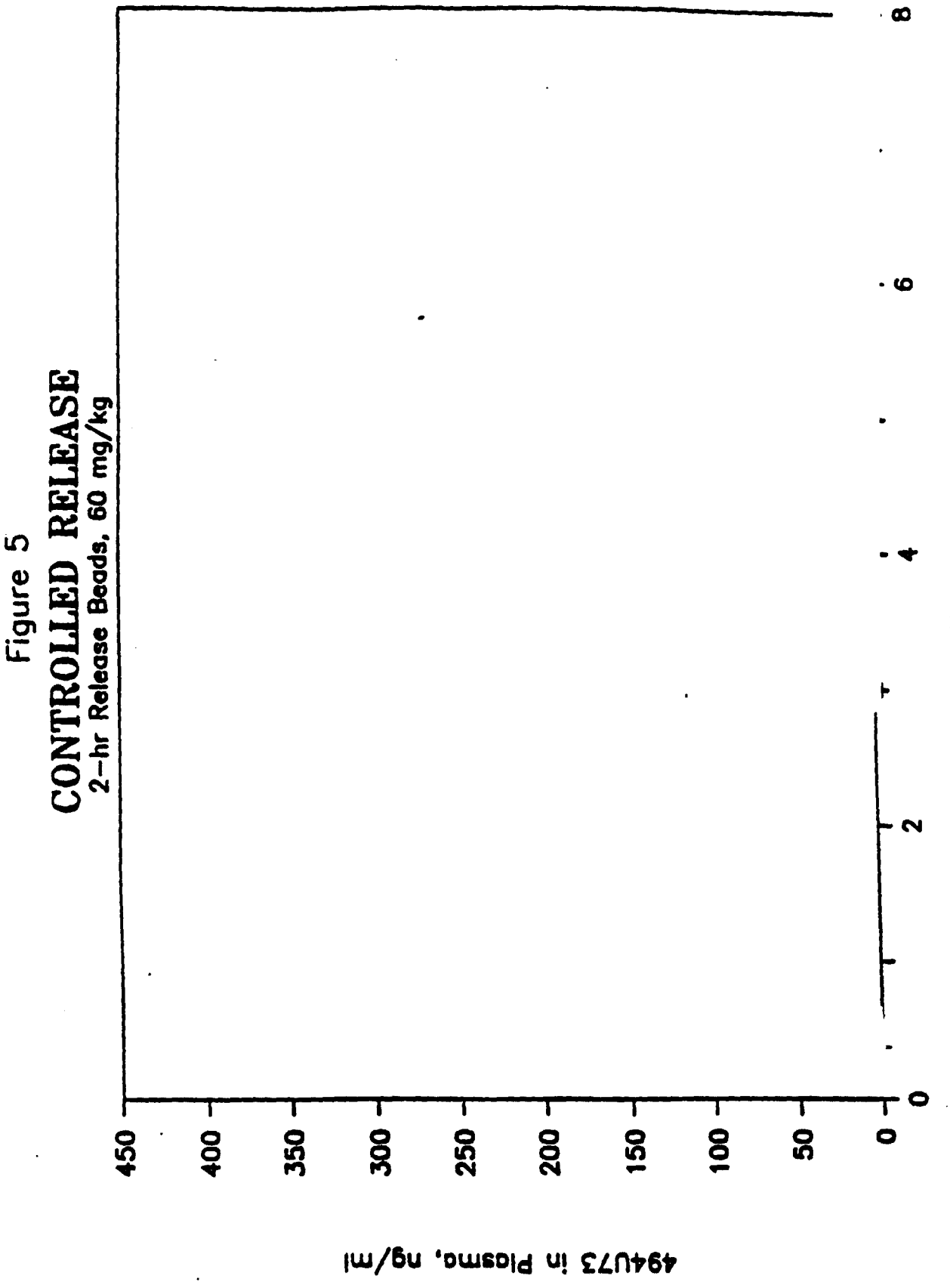
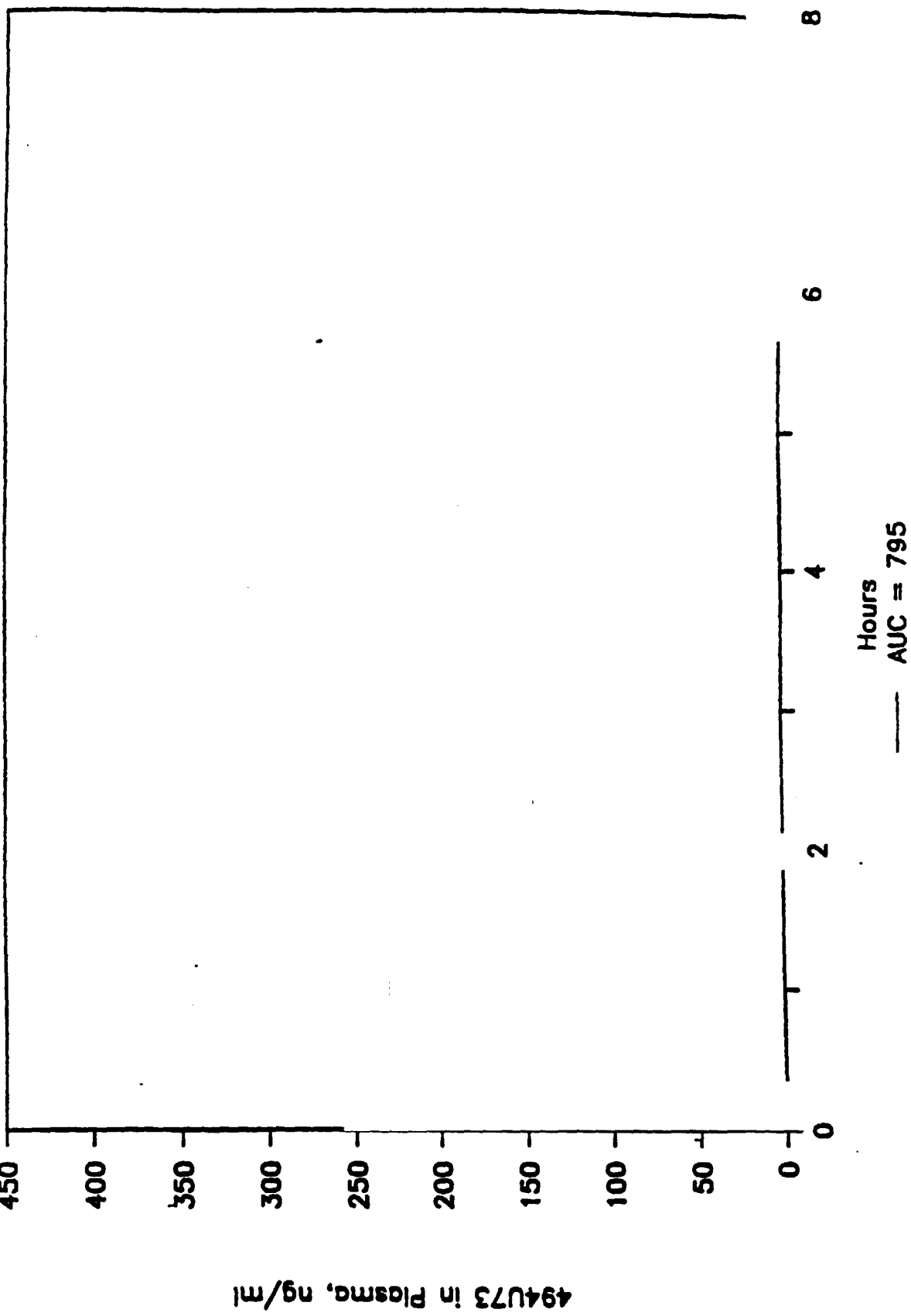

CONTROLLED RELEASE
2-hr Release Beads, 60 mg/kg
494U73 in Plasma, ng/ml
450
400
350
300
250
200
150
100
50
0
0
2
4
6
8
Hours
AUC = 878

Figure 6


INSTANT RELEASE
Dist. Water Soln., 60 mg/kg
494U73 in Plasma, ng/ml
450
400
350
300
250
200
150
100
50
0
0
2
4
6
8
Hours
AUC = 795

2) Page 5-6 ("Metabolism")

a) Third paragraph

The statement that B induces its own metabolism in animals was included in the original labelling. However, data in the newly submitted 90 day rat study appears to contradict earlier data in that plasma levels of B increased with increasing duration of administration. Furthermore, the animal results are now somewhat irrelevant in that B has apparently subsequently been shown not to induce its own metabolism in humans, as stated in this paragraph. The first sentence should therefore be deleted.

b) Fourth paragraph

The pharmacological activity of the metabolites is discussed (structures shown on attached page). The reference to this data is the submission (to NDA 18-644) of 3/11/93, which contains an antitetrabenazine study in mice. (Further data, not referenced here, are summarized in a review paper submitted to NDA 18-644 on 1/28/93; it is not clear if the studies on which these data are based have ever been submitted). The results of the anti-tetrabenazine study are shown in the attached table. (It is not clear what "ED_{50}" means in that criterion for a positive response was not given; it was stated that "test compound scores were converted to a percent of the rating for positive controls [amitriptyline] corrected for a rating received by mice given vehicle." Also note that no other results were shown aside from those shown in the table, e.g., results at each dose level, indications of variation, and statistical analyses). The relative potency of the metabolites (compared to parent) in the proposed labelling is based on the "ED_{50}" values obtained in this study. I believe that it is misleading to give these specific potency comparisons for the following reasons:

1) Comparisons of potency using in vivo dosing of parent and metabolite are problematic in general, e.g., the ADME/PK of the metabolite when administered to the animal may be different from that occurring when the metabolite is formed in vivo, and the potency comparison is not really between just the parent and metabolite but between the parent plus metabolite (formed in vivo) and metabolite.

2) Using the results of a single study at a single time point in a single sex in a single species in a single model of depression is not sufficient to make a sweeping statement about relative potencies. Furthermore, it ignores other data which do not always show similar results, e.g.

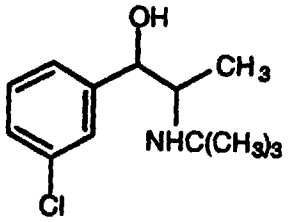
a) As summarized in the submission of 1/28/93,

Table 1. The Effects of Bupropion and Three of its Major Metabolites in the Antitetrabenazine (Anti-TBZ) and Lethal Effects (LD_{50}) in the Mouse After Intraperitoneal Administration

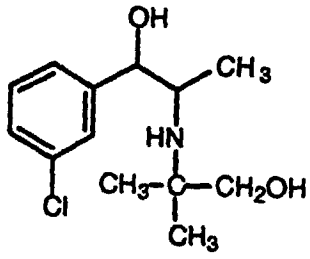
Compound	Anti-TBZ ED_{50} mg/kg	LD_{50} mg/kg
Bupropion	12.5	230
306U73	22	95
494U73	60	135
17U67	60	150
Amitriptyline	4	75

(I.P. dosing)

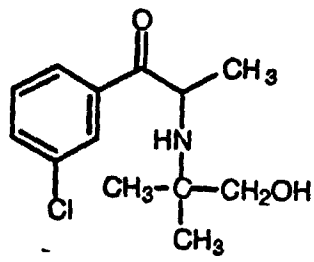
Plasma and urinary metabolites include biotransformation products formed via reduction of the carbonyl group and/or hydroxylation of the *tert*-butyl group of bupropion. Four basic metabolites have been identified. They are the *erythro*- and *threo*-amino alcohols of bupropion (17U67 and 494U73, respectively), the *erythro*-amino diol of bupropion (287U73), and a morpholinol metabolite (306U73).



17U67 (Erythro)
494U73 (Threo)



287U73



306U73

metabolite 306U73 was 2 times more potent than the parent in decreasing noradrenergic firing rate in rats (and metabolite 494U73 had no effect at a dose about 5 x that of the parent). It was also stated that B, although not metabolites 306U73 and 494U73, decreased dopaminergic firing rate when given i.v., but the doses tested were not stated.

b) As shown in the attached table taken from the submission of 1/28/93, metabolites 306U73 and 494U73 ranged from being approximately equipotent to being 20x less potent than B in blocking neurotransmitter uptake in vitro. (However, note that potencies were relatively low in all cases).

c) The i.p. LD_{50} values for 3 metabolites were lower than that of parent compound in mice. (Values included in the table of anti-tetrabenazine results).

Thus, the relative potency values of parent and metabolites given in the proposed labelling are misleading; in fact I do not think it warranted even to imply that the metabolites are less potent than the parent compound based on the data provided. A more accurate picture might be to leave out the statements concerning relative potency (e.g., from "however" on line 114 to "bupropion" on line 116) so that this section might read: "...These metabolites of bupropion are pharmacologically active although their potency and toxicity relative to bupropion have not been fully characterized. They may be of clinical importance because their plasma concentrations are higher than those of bupropion." (I have left out the phrase "in chronic use" from the last sentence; are these metabolites not quantitatively significant in acute or subacute use?).

TABLE 5. IC_{50} Values for Bupropion, 306U73, and 494U73 as Inhibitors of ^3H-Biogenic Amine Uptake into Synaptosomal Preparations of Various Rat and Mouse Brain Areas.

		IC_{50} Values (mean ± SE)[a]		
		Hypothalamus		Striatum
Compound	Species	^3H-ℓ-NE (μM)	^3H-5HT (μM)	^3H-DA (μM)
Bupropion	Rat	5 ± 1.3	58 ± 15	2 ± 0.58
	Mouse	4.4 ± 0.75	36 ± 21	2.5 ± 0.82
306U73	Rat	6.6 ± 3.3	105 ± 11	23 ± 8.3
	Mouse	3.6 ± 1.2	100 ± 11	17 ± 9
494U73	Rat	16 ± 7.2	67 ± 2.1	47 ± 2.2
	Mouse	10 ± 13	92 ± 15	23 ± 13
Imipramine	Rat	.32 ± .11	.5 ± .04	58 ± 12
	Mouse	.10 ± .03	.45 ± .24	56 ± 11

[a] Drugs were incubated in presence of the tissue for 5 minutes at 37°C before the addition of 1 x 10^{-7}M ^3H-l-NE, 1 x 10^{-7}M ^3H-DM or 1 x 10^{-7}M ^3H-5HT. Incubation was continued for an additional 5 minutes at 37°C in an atmosphere of 95% O_2 - 5% CO_2. A minimum of 3 and in most cases 5 different concentrations of the drug producing 30%-70% inhibition of uptake of the biogenic amines were used to determine the IC_{50} values. The results were plotted on semilogarithmic paper.

3) Page 15-16 ("Carcinogenesis, Mutagenesis, Impairment of Fertility")

The sponsor wishes to change the statement that bupropion was positive in a cytogenetic study in rats to say that it was only positive in 1 of 3 studies. I am only aware of 2 studies (1 reviewed under NDA 18-644; the other reviewed above; bupropion was positive only in the former); the sponsor should identify the 3rd study. I am not sure if characterizing the effect as "weak" in the positive study is correct; an increase in chromosomal aberrations of 2-3x control was seen. The route of administration (oral) should be stated. A revised statement might read: "Bupropion caused an increase in chromosomal aberrations only at the highest dose (300 mg/kg/day p.o.) in one of two [or three if documented by sponsor] rat cytogenetic studies."

4) Page 28 ("Lethal Doses in Animals")

Although the sponsor has not requested a change in this section, the utility of giving animal LD_{50} values and toxic signs is not clear. (The production of seizures in animals and humans is discussed elsewhere in the labelling).

EVALUATION:

The sponsor has adequately performed the preclinical studies requested by us and this NDA is approvable. The findings were generally similar to those in studies performed under the NDA for the immediate release formulation (NDA 18-644). (One exception to this was a lack of an increase in chromosomal aberrations in the new rat study). In studies where they were compared, there were no clear differences between "undegraded" and "degraded" (i.e., containing degradation products/impurities at or above their limits in the human sustained release formulation) bupropion. (Particular attention was paid to effects on the liver, which was identified as the primary target organ for toxicity in the original studies. Since long term administration of bupropion caused hyperplastic nodules in rat liver, it was deemed important to compare the early effects of "degraded" and "undegraded" bupropion in the 90 day rat study. Both preparations caused increased in liver weights and hepatocellular hypertrophy with no apparent differences between them).

It is noted that whereas the sponsor listed the amounts of impurities in the "degraded" bupropion which was tested, the amounts of impurities in the "undegraded" bupropion were not given. Since a crucial concern in these studies was to compare the results obtained with the "new" bupropion with the bupropion used in the original preclinical studies (performed under NDA 18-644) it is important that the "undegraded" bupropion tested in the comparative studies be the same or very similar to that used in the original studies. The sponsor should be requested to verify this.

The proposed labelling contains numerous differences from the labelling originally approved for the immediate release formulation under NDA 18-644. My comments on the sections based on animal data are discussed above under "Labelling". (Note that most of the proposed animal data-based changes are not specific to the sustained release formulation and would thus presumably also apply to the labelling of the immediate release formulation.) I have not been able to locate the studies used to support some of the proposed changes; the sponsor should be requested to submit (or indicate if and when previously submitted) these studies (see "Recommendations" section for specifics).

RECOMMENDATIONS:

This NDA is approvable provided the sponsor verifies that the "undegraded" bupropion used in the studies performed contains amounts of impurities similar to those present in the drug which was originally used in the pivotal preclinical studies performed under NDA 18-644.

The sponsor should also be requested to submit (or indicate if and when submitted) the following studies which are being used to support proposed labelling changes: (1) the study on effects of bupropion and its metabolites on firing rates of noradrenergic, dopaminergic, and serotonergic neurons in rat brain and (2) the "third" rat cytogenetic study (I am only aware of 2, i.e., the one originally submitted under NDA 18-644, which was positive, and the one submitted with the present NDA, which was negative).

My comments on the proposed labelling revisions are made above under "Labelling".

Barry Rosloff

Barry N. Rosloff, Ph.D.

cc: NDA 20-358
HFD-120
HFD-120/GFitzgerald
/BRosloff
rd/ejs/9/30/94
ft/ejs/10/7/94
N:\Rosloff/NDA20358

GFF 11/1/94
/P. David