CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-711

PHARMACOLOGY REVIEW(S)

CENTER FOR DRUG EVALUATION AND RESEARCH

DIVISION OF ANESTHETIC, CRITICAL CARE AND ADDICTION DRUG PRODUCTS

PHARMACOLOGY REVIEW

NDA #: NDA 20-711

SPONSOR: Glaxo Wellcome Inc.
Research Triangle Park, N.C.

INFORMATION TO SPONSOR: Yes (x) No ( )

DATES:

SUBMISSION:	May 17, 1996
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REVIEWER: BeLinda A. Hayes, Ph.D.
CSO: Bonnie McNeal

PRODUCT NAME:	ZYBAN™
GENERIC NAME:	Bupropion Hydrochloride
CHEMICAL NAME:	(±)-1-(3-chlorophenyl)-2-[1,1-dimethylethyl)amino]-1-propranone hydrochloride
CAS NUMBER:	31677-93-7; BW 323U
CLINICAL FORMULATION:	Sustained-Release Tablet
CLINICAL DOSAGE & ROUTE OF ADMINISTRATION:	300 mg/day (150 mg b.i.d.), Oral
DOSAGE FORM:	Sustained Release Tablets
CLINICAL STRENGTH:	50, 100, and 150 mg strengths
DRUG CLASS:	Anti-depressant
INDICATIONS:	Aid to smoking cessation
RELATED INDs:	
RELATED NDAs:	18-644; 20-358

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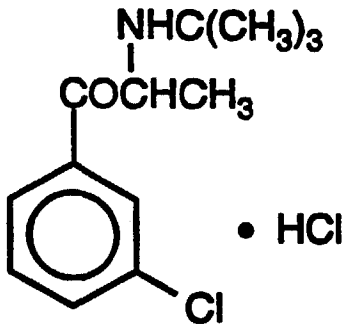
DRUG SUBSTANCE.

Bupropion hydrochloride is a white crystalline powder which is freely soluble in water. It is bitter tasting and will produce the sensation of local anesthesia on the oral mucosa.



Empirical Formula: $C_{13}H_{18}ClNO \cdot HCl$ Molecular Weight: 276.2 g/mole

CLINICAL FORMULATION OF BUPROPION TABLETS:

COMPONENT	50 MG TABLETS (WHITE)	100 MG TABLETS (BLUE)	150 MG TABLETS (PURPLE)
Bupropion HCl	mg	mg	mg
Microcrystalline Cellulose NF	mg	mg	mg
Hydroxypropyl Methylcellulose 2910 USP	mg	mg	mg
Cysteine HCl USP	mg	mg	ng
Magnesium Stearate NF	mg	ng	ng
Tablet Core Weight	mg	mg	mg
COATING			
Carnauba Wax NF	mg	mg	mg
	mg		
		mg	
			mg
Coated Tablet Weight	mg	mg	mg

INTRODUCTION/DRUG HISTORY.

Bupropion hydrochloride (WELLBUTRIN®), (±)-1-(3-Chlorophenyl)-2-[1,1-dimethylethyl)amino]-1-propranone hydrochloride, an aminoketone derivative which is structurally related to phenylethylamine was originally developed by Glaxo Wellcome Inc. as an antidepressant. The immediate-release formulation of bupropion hydrochloride was approved by the Division of Neuropharmacological Drug Products on December 30, 1985 (NDA 18-644). It was marketed in 1989 for the treatment of depression under the proprietary name of WELLBUTRIN®. The recommended therapeutic dose was in the range of 300 to 450 mg per day at a t.i.d. dosing interval

Subsequently, Glaxo Wellcome Inc. developed a sustained-release formulation of bupropion (WELLBUTRIN SR) with the intent of achieving a b.i.d. dosing schedule and reducing the incidence of the adverse effect associated with the t.i.d. dosing schedule. Agitation, insomnia, dry mouth, headache/migraine, nausea/vomiting, constipation and tremors are the most common adverse effects associated with the use of WELLBUTRIN. Of the utmost concerns are the major motor seizures that has been reported in some patients at doses up to 450 mg/day. The sponsor has demonstrated that WELLBUTRIN SR is bioequivalent to the immediate-release formulation of bupropion. FDA's Division of Neuropharmacological Drug Products issued an approvable letter on March 12, 1996 to Glaxo Wellcome for their pending WELLBUTRIN SR NDA 20-538. Glaxo Wellcome received an approval letter for Wellbutrin SR, NDA 20-538 on October 4, 1996.

Pending in the Division of Anesthetic, Critical Care and Addiction Drug Products is an NDA for WELLBUTRIN SR as an aid for smoking cessation. The concept to study the efficacy of bupropion as an adjunctive therapy for smoking cessation is the "brain storm" of Dr. Linda Ferry. Dr. Linda Ferry was inspired by some anecdotal data from clinical trial studies that showed some depressed patients spontaneously ceased smoking while being treated with WELLBUTRIN. Preliminary findings in two placebo-controlled studies positively demonstrated that WELLBUTRIN at a dose of 300 mg/day was safe and effective as an aid to smoking cessation. Based on these results, Glaxo Wellcome Inc. initiated their clinical development program for the use of WELLBUTRIN SR as an adjunct therapy for smoking cessation.

FOREIGN MARKETING HISTORY. Bupropion immediate-release and bupropion sustained-release tablets have not been approved or marketed in any foreign countries. However, an application for marketing approval of the immediate-release formulation, for the treatment of depression, was submitted in the United Kingdom (UK) and Canada

NEW PRECLINICAL STUDIES SUBMITTED:

No new preclinical studies were submitted in this NDA submission. Preclinical toxicology studies supporting the safety of the marketed immediate release formulation of bupropion was reviewed under NDA 18-644 (Original Summary of 6/15/82) by Barry Rosloff, Ph.D. a reviewing Pharmacologist in HFD-120. Preclinical toxicology studies supporting the safety of the sustained release formulation was reviewed under NDA 20-358 (Original Summary of 9/24/94) by Barry Rosloff. His reviews are located in Appendices 1, 2 and 3.

Studies Reviewed Within This Submission. None

Studies not reviewed within this submission. None

Impurities/Degradation Products. The sustained release formulation of bupropion contains two new degradation products with a proposed limit of 2% each, and several other degradation products/impurities with limits ranging from 1 to 5% each. The total limit of these impurities will be 8% (this will represent 24 mg/day at the maximum recommended dose of 300 mg in humans). To demonstrate that these degradation products and impurities will pose no significant risk to man, HFD 120 had the sponsor conduct several bridging studies with drug containing the maximum allowable amounts of impurities in the new formulation. The studies performed were: 1) 90 day p.o. toxicity in rats; 2) Segment II reproductive study in rats; 3) Ames Test; and 4) *In vivo* cytogenic study in rats. These studies were reviewed by Barry Rosloff in his NDA review of 9/24/94 (Appendix 3).

PHARMACOLOGY

Bupropion, (±)-1-(3-Chlorophenyl)-2-[1,1-dimethylethyl)amino]-1-propranone hydrochloride, an atypical antidepressant with a distinct neuropharmacological and behavioral profile. In contrast to the typical antidepressants, bupropion does not desensitize α- and β-adrenergic and sertoninergic receptors; it does not affect catecholamines release from synaptosomes and monoamine oxidase activity. It has been reported that bupropion mediates some of its effects via the dopaminergic system. *In vitro* and *in vivo* studies have shown that bupropion is a selective inhibitor of dopamine uptake (Cooper *et al* , 1980; Ferris *et al* 1983; Richelson and Pfenning, 1984; Marek *et al.*, 1990; Deresch *et al.*, 1994). Microdialysis studies have demonstrated that bupropion significantly increases dopamine extracellular concentrations in the striatum and nucleus accumbens.

One may hypothesize that bupropion may be attenuating nicotine withdrawal symptoms and reducing craving through its actions on the dopaminergic system. It is well documented that the addictive effects (i.e., reinforcing effects) of nicotine are the results of its effects on the dopamine system in the mesolimbic system. Nicotine and other drugs of abuse produce their rewarding or reinforcing effects by stimulating the release of dopamine from dopaminergic neurons within the mesolimbic system. Nicotine stimulates the release of dopamine by binding to nicotinic receptors located on the cell bodies of these dopaminergic neurons. Supporting evidence that nicotine's reinforcing effects are centrally mediated and involve dopamine include: 1) Attenuation of nicotine's reinforcing effects in primates pretreated with the nicotinic antagonist mecamylamine (Goldberg *et al.*, 1981); 2) Administration of dopamine antagonists blocks the reinforcing effects of nicotine in rats (Clarke, 1990; Corrigall and Coen, 1991); 3) Attenuation of nicotine's reinforcing properties after infusions of 6-OHDA, a dopaminergic neurotoxin, into the nucleus accumbens of rats trained to self-administer under a FR 5 schedule (Corrigall *et al.*, 1992); 4) Reduction of the number of nicotine binding sites as well as weakening of self-administration in rats after lesion of the mesolimbic dopaminergic system (Singer *et al.*, 1982; Clarke *et al.*, 1988; Clarke, 1990).

The pharmacological profile of bupropion has been reviewed in details by Barry Rosloff of Division of Neuropharmacological Drug Products. See his review in Appendix 1.

TOXICOLOGY STUDIES

TOXICOLOGY

The potential toxicity of bupropion has been evaluated in several species following single and repeated administration. These toxicity studies were originally submitted under IND and reviewed by HFD 120. The toxicity studies performed are summarized in Table 1.

The acute toxicity of bupropion was assessed in CD-1 mice, CD rats (neonatal, immature, and adults) and Long-Evans rats following oral and/or intraperitoneal administration. These studies were reviewed in detail by Barry N. Rosloff, Ph.D. The acute oral toxicity studies (Study Report Nº TTEP/82/0091) conducted in CD rats were reviewed on June 15, 19982; and the studies conducted in CD-1 mice and Long-Evans rats were reviewed on October 29, 19982. Dr. Rosloff's detailed reviews are located in Appendix 1 (June 15, 1982 review) and Appendix 2 (October 29, 1982). In brief, the major findings of these studies are summarized in Table 1. As depicted in the Table 1, bupropion-induced overt signs of toxicity were observed in mice and rats following acute intraperitoneal or oral administration. Clinical signs which were common to each species included: ataxia, convulsions, prostration, ptosis, labored breathing, salivation, and compulsive gnawing. In most instances, bupropion-induced death occurred on the day of dosing; some delayed deaths (2 days or 4-5 days post-dosing) were also observed. The cause of death was not established.

Subchronic toxicity studies were conducted in rats and dogs using the intended oral route of administration. These toxicity studies were originally submitted under NDA 18-644 and reviewed by HFD-120 on June 15, 1982. The NDA review is included in Appendix 1. A summary of the clinical signs resulting from bupropion treatment for 14, and 90 consecutive days are presented in Table 1.

In the 12 week subacute toxicity study (Study Report Nº. TTEP/70/0009), Long Evans rats (10 ♂ and 10 ♀) received daily dosages of 0, 150, 300 or 450 mg/kg/day by gavage (for the first 12 days, doses were 100, 200, and 300 mg/kg in the low dose, medium dose and high dose group, respectively). Bupropion-induced mortality were observed in one rat treated with 150 mg/kg and 2 rats treated with 300 mg/kg and 450 mg/kg. Observed clinical signs included: dose-dependent urinary retention, blood occurring in urine after 5 weeks of treatment, and irritability. Relative to the control, the SGOT value was lower in males dosed with 300 and 450 mg/kg group. A statistically significant increase in total serum protein was observed in both males and females in the 450 mg/kg group. Relative to the controls, organ to body weight ratio analysis revealed a statistically significant increase in the liver of the rats treated with bupropion. Histopathological analysis showed that the livers of the bupropion-treated rats showed a increase in cell multiplication and the presence of cytoplasmic organelles. No treatment-related body weight change was observed in the bupropion treated rats.

In the dog subchronic toxicity study (Study Report Nº. TTEP/70/0001), 2 male and 2 female Beagle dog received daily oral doses of bupropion at dose levels of 0 (200 mg lactose), 15 mg/kg, 35 mg/kg, and 75 mg/kg (at day 45, the dose was increased to 150 mg/kg). No drug-induced deaths occurred. Also, no bupropion-related effects were observed on body weight or clinical observations. Selected hematology, serum biochemistry and urinalyses parameters evaluated were within normal range. There were no bupropion-related histopathological changes observed during necropsy. However, relative to the control group, the absolute and relative weight of the liver of the dogs in high dose group was slightly increased (18% above control).

Due to the presence of several impurities/degradation products in the sustained release formulation of bupropion, a 90 day oral toxicity study (Study Report Nº. TTEP) was performed in Charles River CD rats to determine the toxicopathological effects of these degradation products. The bupropion used in this study contained the following impurities: 3-chloro-benzoic acid (0.7%), 26W93 (2.7%), 3134W92 (0.8%), 26W93 (1.8%), 852U77 (6.2%), 20U78 (2.4%), and 827U76 (1.3%). The presence of these impurities did not alter the toxic profile of bupropion. The findings in this study did not differ from those observed in studies TTEP/70/0009 and TTEP/92/0011. The daily administration of bupropion (containing impurities) produced salivation and convulsions in a dose-dependent manner. A dose-dependent increase in liver weights and thyroid weight were noted. These effects were related to microsomal enzyme induction and were reversible. Also, the histopathological effects of 300 mg/kg/day bupropion (containing impurities) and 300 mg/kg/day (without impurities) were similar. Both produced centrilobular hepatocellular hypertrophy.

The toxicity associated with chronic (52 wks in rats; 55 wks. in dogs) exposure to bupropion was assessed in rats and dogs following oral administration. In the rat study, 60 male and 60 female Charles River CD® rats were administered bupropion daily by intubation at dosage levels of 0 (untreated), 0 (vehicle), 25, 50, and 100 mg/kg/day. No treatment-related deaths occurred. There were no significant overt signs of toxicity seen throughout the study in the bupropion treatment groups. However, it should be noted that a yellow staining of the hair around the anogenital area was prevalent in the treatment group; this observed sign was dose-dependent. Also, the presence of a dry brown material was noted around the mouth or nose, and moisture around the mouth was also observed in the treated rats. Males in the high-dose group exhibited a slight decrease in body weight gain. Some serum biochemistry parameters were affected by bupropion treatment. The glucose level for most rats treated with 100 mg/kg/day was slightly lower (15 % lower) than the control rats. One female in the mid-dose group and two females in the high-dose group had elevated BUN levels. The SGOT and SGPT levels were higher than control values in one female and one male dosed with 25 and 50 mg/kg/day, respectively.

Relative to untreated rats, a statistically significant differences were evident in the absolute and/or relative weight of the liver and kidney of bupropion-treated rats. Both the absolute and relative weights of the liver were higher than the control in females treated with 25, 50 and 100 mg/kg of bupropion. The relative weight of the livers of the males (50, and 100 mg/kg/day) was increased relative to the untreated control. The weights (relative) of the kidney the males (50 and 100 mg/kg) and brain of the males (vehicle control and 100 mg/kg) was statistically higher than the untreated control. Heart weight and thyroid/parathyroid weight were significantly decrease in females at 100 mg/kg/day and 25 mg/kg/day, respectively. Histopathological analysis revealed a increase in the amount of hemosiderin detected in the spleen of male rats in the 100 mg/kg/day group.

Two carcinogenicity studies were conducted in rodents; a 23-24 month study (Study Report Nº. TTEP/80/0096) in rats and a 21-22 month study (Study Report Nº. TTEP/80/0095) in mice. Both studies were submitted in NDA 18-644 and were reviewed by Barry Rosoloff in HFD-120. Dr. Rosoloff reported that there were no drug-related increases in either total benign or malignant tumors (corrected for increased mortality in drug groups by use of life-table analyses) or in any specific tumor type in both the mouse and rat carcinogenicity studies (See detail review located in Appendix 1). However, in the rat carcinogenicity study there were some drug-induced changes in the liver. There was an increase in incidences of hyperplastic nodules and hepatocellular hypertrophy at all doses (100, 200, and 300 mg/kg/day by gavage) of bupropion and an increase incidence in hepatocellular hyperplasia in the low (100 mg/kg/day) and mid (200 mg/kg/day) dose groups.

Table 1. Summary of Toxicity Studies With Bupropion.

SUMMARY OF ACUTE TOXICITY STUDIES					
STUDY Nº	**STUDY CATEGORY**	**ROUTE**	**DOSAGES (MG/KG)**	**SPECIE**	**SIGNIFICANT FINDING(S)**
TTEP/78/0002	LD_{50} Determination	IP, PO	IP: 200, 25, 250, 275, and 300 PO: 400, 500, 600 and 700	Mice (CD-1)	IP: • Dose-dependent overt signs of toxicity were observed. • Overt signs included: ataxia, clonic convulsions or opisthotonos followed by prostration, labored breathing, ↓ respiration, salvation, ptosis, and compulsive gnawing. • LD_{50}: ♂ = 273 mg/kg PO: • Overt signs of toxicity included: ataxia. prostration, clonic convulsions, ptosis, and compulsive gnawing. • Death occurred at all doses from 5 min to 2 days post-treatment. • LD_{50}: ♂ = 544 mg/kg; ♀ = 636 mg/kg
TTEP/78/003	LD_{50} Determination	IP, PO	IP: 175, 200, 225, and 275 PO: 400, 500, 500, 600 and 700	Rats (Long-Evans)	PO: • Overt signs of toxicity included: ataxia, loss of righting reflex, labored breathing, prostration, salivation, ptosis, arched back, and compulsive gnawing. • LD_{50}: ♂ = 263 mg/kg; death occurred 1-22 hr post-dosing. Some delayed (4-5 days) death did occur. IP: • 175 mg/kg = NOEL • Overt signs of toxicity included: ataxia, loss of righting reflex,labored breathing, prostration, salivation, ptosis. arched back, and compulsive gnawing. • LD_{50}: ♂ = 607 mg/kg; ♀ = 482 mg/kg; death occurred within 1-25 min after dosing.

SUMMARY OF ACUTE TOXICITY STUDIES					
STUDY N°	TEST TYPE	ROUTE	DOSAGES (MG/KG/DAY)	SPECIE	SIGNIFICANT FINDING(S)
TTEP/82/0091	LD_{50} Determination	PO	0, 150, 250, 350, and 450	Rats (CD; 6 days old)	• No overt signs of toxicity observed. • Most death occurred on day of dosing. • LD_{50}: ♂ = 469 mg/kg; ♀ = 499 mg/kg.
TTEP/82/0091	LD_{50} Determination	PO	0, 300, 400, 500, and 600	Rats (CD; 17 days old)	• ↓ activity and ataxia were observed at doses of 400 to 600 mg/kg. • Most deaths occurred on the day of dosing. • LD_{50}: ♂ = 739 mg/kg; ♀ = 763 mg/kg
TTEP/82/0091	LD_{50} Determination	PO	200, 300, 400, and 500	Rats (CD: 27 days old)	• dose-dependent overt sings were observed. • 200-500: salivation, ataxia, preconvulsive seizures, clonic convulsions. • 400-500: loss of righting reflex, prostration, ptosis, gnawing. • Death occurred on day 1 of dosing through 3 days post-dosing. • LD_{50}: ♂ = 678 mg/kg; ♀ = > 666 (no deaths at this dose)
TTEP/82/0091	LD_{50} Determination	PO	550, 650, 750, and 850	Rats (CD: 39 days old)	• dose-dependent overt sings were observed. • 550-850: salivation, ataxia, preconvulsive seizures, clonic convulsions. • 650-850: clonic and/or tonic seizures, and labored breathing • Death occurred on day 1 of dosing through 3 days post-dosing; most of the death was observed in the females. • LD_{50}: ♂ = 683 mg/kg; ♀ = 342 mg/kg

SUMMARY OF REPEATED DOSE TOXICITY STUDIES					
STUDY N°	**TEST TYPE**	**ROUTE**	**DOSAGES (MG/KG/DAY)**	**SPECIE**	**SIGNIFICANT FINDING(S)**
TTEP/92/0011	14 Day	PO	0, 100, 200, and 300	Rat (CD)	• A reversible dose-dependent ↑ in absolute and relative liver wt. (≈ 5% -30%) in both ♂ & ♀. • The effect on the liver was due to microsomal enzyme induction.
TTEP/70/0009	12 weeks	PO	0, 100, 200 and 300 for first 12 days in LD, MD, and HD, respectively. 0, 150, 300, and 450 for 78 days	Rat (Long Evans)	• No specific bupropion-induced toxicity were at doses up to 450 mg/kg/day. • An significant ↑ in serum protein was observed in both ♂ and ♂ rats receiving 450 mg/kg/day. • A dose-dependent ↑ in liver weight. • SGOT was significantly ↓ in ♂ rats receiving the middle and high dose of bupropion.
TTEP/93/0061	90 Day	PO	0, 75, 150, 300, and 300 (without impurities)	Rat (CD)	• Dose-dependent ↑ salivation. • ↑ liver absolute and relative weights (all doses). • Centrilobular hepatocellular hypertrophy in both high dose groups. • Slight ↑ in thyroid and adrenal wt. in high dose groups. • ↑ incidence of chronic progressive nephrosis in ♂.
TTEP/76/0031	6-Month		0, 100, 200 and 300 (this group received 500 from day 1-15 and 400 from day 16-74)	Rat (CD)	• Statistically significant ($p<0.05$) ↑ in group (300 mg/kg/day; ♂ and ♀) mean liver weight. • Statistically significant ↑ in group mean liver weight in ♂ & ♀ at 100 mg/kg/day ($p<0.05$), ♂ at 200 mg/kg/day ($p<0.01$) and ♂ & ♀ at 300 mg/kg/day ($p<0.01$).
TTEP/78/0070	55-Weeks	PO	0, 25, 50, and 100	Rat (CD)	• The absolute and relative wt. of the liver was increased. • The absolute and relative wt. of the kidney was increased in all groups. • ↑ amt. of hemosiderin pigment in the spleen, liver and lung. • ↑ incidence of focal chronic nephritis.

SUMMARY OF REPEATED DOSE TOXICITY STUDIES					
STUDY Nº	**STUDY CATEGORY**	**ROUTE**	**DOSAGES (MG/KG)**	**SPECIE**	**SIGNIFICANT FINDING(S)**
TTEP/70/0001	90 Day	PO	0, 15, 35, AND 150 (received 75 mg/kg/day from day 1-45 and 150 mg/kg from day 46-92)	Dog (Beagle)	• The absolute and relative wt. of the liver of the HD group was slightly increased (relative wt. 18 above control)
TTEP/78/0001	52-Weeks	PO	0, 40, 80, and 150 (received 80 mg/kg/day from weeks 1-3, 120 mg/kg/day week 4; 150 mg/kg weeks 5-52)	Dog (Beagle)	• 2 ♀ and 1 ♂ died 150.0 mg/kg group • dose-related ↑ of serum alkaline phosphatase in all group. • ↑ SGOT and SGPT in 150 mg/kg group. • Dose-dependent ↑ in liver weight • Drug-induced histological changes in the liver and kidney. • Increase in kidney weight in all groups.
SUMMARY OF CARCINOGENICITY STUDIES					
TTEP/80/0096	Carcinogenicity (23-24 months study)	PO	0, 100, 200, and 300	Rat (CD)	• Increase incidence of hepatocellular hyperplasia in the low and mid dose group. • Increase incidences of hyperplasia nodules and hepatocellular hypertrophy at all doses. • No drug-related increases in either total benign or malignant tumors.
TTEP/80/0095	Carcinogenicity (21-22 month study)	PO	0, 50, 100, and 150 - All animals were initiated at 50 mg/kg/day and were ↑ over a 6 wk. period to final dose level	Mice (CD-1)	• No drug-related increases in either total benign or malignant tumors.

REPRODUCTIVE AND TERATOLOGY STUDIES

REPRODUCTIVE TOXICITY STUDIES.

The reproductive toxicity potential of bupropion was evaluated in rats and rabbits. Its effects on fertility and reproductive performance were assessed in rats following oral administration. Bupropion effects on peri-, postnatal, and neonatal development were evaluated in rats. The teratogenic potential of bupropion was studied in both rats and rabbits. The reproductive studies conducted and reviewed in details by Barry Rosloff, Ph.D. in HFD-120 are listed in Table 2.

Based on the reviews of Dr. Barry Rosloff, bupropion is not a reproductive toxicant in Long-Evan rats. Fertility, Embryofetal Developmental studies in rats demonstrated that bupropion had no direct effect on reproductive performance or fertility; that is duration of gestation, duration of parturition, pregnancy rate, and implantation were not affected by bupropion treatment. The number of live pups born in the low and medium dose groups were not affected by bupropion treatment. However, in comparisons to the vehicle treatment group, the mean number of live pups in the high dose group was slightly decreased. No treatment-related malformations were evidence in the F_1 rats.

The teratology study (Study Report Nº. TTEP/93/0062) in CD® rats demonstrated that the presence of the degradation products/impurities in the sustained-release formulation of bupropion did not alter the reproductive toxicity profile of bupropion. Bupropion containing 8% degradation products/impurities had no effect on the reproductive performance or fertility in CD® rats. Dr. Rosloff reported that bupropion (containing impurities) at doses up to 300 mg/kg/day was without teratogenic or embryocidal effects; no significant effects on the numbers of corpora lutea, the numbers of implantation sites, live fetuses, pre- and post-implantation losses, sex ratio, fetal bodyweight, external, visceral, or skeletal anomalies/malformations were observed. However, it was reported that both 300 mg/kg/day treatment group caused a slight decrease in the pregnancy rate; relative to the control (95%) the pregnancy was approximately 85%.

The EmbryoFetal Developmental Study (Study Report Nº. TTEP/76/006) performed in pregnant rats suggested that bupropion does not induce any embryocidal and teratogenic effects following oral administration. There was no bupropion-induced effects on gross or visceral fetal abnormalities observed. However, a slight increase in incidence of reduced or absent ossification of some bones were observed in fetus of the 300 and 400 mg/kg/day groups. Also, it was reported that a slight decrease in fetal weight and length was noticed in all bupropion groups.

In contrast, bupropion-induced skeletal abnormalities were observed in New Zealand White rabbits. Increase number of fetus with supernumerary ribs and delayed ossification were noted.

TABLE 2. Reproductive Studies Performed to Evaluate the Reproductive Toxicity Potential of Bupropion.

SUMMARY OF REPRODUCTIVE TOXICITY STUDIES WITH BUPROPION					
STUDY №	**TEST TYPE**	**STUDY TITLE**	**ROUTE**	**DOSES (mg/kg/day)**	**SPECIE**
TTEP/76/003	Fertility and EmbryoFetal Development	Two-Generation Reproductive and Fertility Study In Long-Evans Rats Given BW 66-323U Orally by Gavage: Phase I.	Oral	0, 100, 200, and 300	Long-Evans Rat
TTEP/76/008	Fertility and EmbryoFetal Development	Two-Generation Reproductive and Fertility Study In Long-Evans Rats Given BW 66-323U Orally by Gavage: Phase I.	Oral	0, 100, 200, and 300	Long-Evans Rat
TTEP/76/0006	EmbryoFetal Development	Teratology Study of BW 323 in Rats.	Oral	0, 150, 300, and 450	Long-Evans Rat
TTEP/93/0062	EmbryoFetal Development	Developmental Toxicity Study in CD® Rats Given 323U66HCl (Containing Impurities) by Gavage.	Oral	0, 10, 75, 150, 300, and 300 (no impurities)	Rats
TTEP/77/003	EmbryoFetal Development	Teratology Study of BW 323 in Rabbits.	Oral	0, 50, 100, and 150	NZW Rabbit
TTEP/77/004	EmbryoFetal Development	Teratology Study of WELLBUTRIN® in Rabbits.	Oral	0, 25, 50, 100, and 150	NZW Rabbit
TTEP/77/0005	EmbryoFetal Development	Summary of Two Teratology Studies of BW 323 in Rabbits.	Oral	0, 25, 50, 100, and 150	NZW Rabbit

MUTAGENICITY STUDIES

The ability of bupropion to cause mutation or chromosomal damage was evaluated in several genotoxictiy assays. The assays performed were the: Ames Test, Rat Bone Marrow Cytogenic Analysis, and Mouse Lymphoma TK Assay.

Ames Test. Bupropion was assayed for mutagenicity in *Salmonella typhimurium strains* TA1537, TA1538, TA98, and TA100 in the presence and absence of Arclor-induced rat liver S9 metabolic activation. Two separate Ames Assays (BWCo Study Reports Nº. TTEP/80/0051, and TTEP/93/065/01). Both studies were conducted in compliance with the Good Laboratory Practice regulations. The first Ames Test (Study Report Nº. TTEP/80/0051) was conducted at during July 1980 to August 8, 1980. The mutagenicity test was performed according to the plate-incorporation procedure of Ames and colleagues (1975). Bupropion was tested over a broad range of concentrations: 60 μg/plate to 6000 μg/plate. Results (Appendix 1) from this Ames Test was reviewed by the Division of Neuropharmacological Drug Products's Pharmacologist Barry N. Rosloff, Ph.D. on June 15, 1982. In brief, Dr. Rosloff reported that Bupropion was weakly positive in the TA100 *Salmonella* strain in both the presence and absence of Arclor-induced rat liver S9 preparation and in the TA1535 strain in the presence of S9 metabolic activation

In the second Ames test, bupropion (plus impurities/degradation products) was assayed for mutagenicity in *Salmonella typhimurium strains* TA1537, TA1538, TA98, and TA100 in the presence and absence of Arclor-induced rat liver S9 metabolic activation. This Ames Test (BWCo. Document Nº.TTEP/93/065/01) was conducted at during September 10, 1993 to November 1, 1993. The mutagenicity test was performed according to the plate-incorporation procedure of Ames and colleagues (1975). Bupropion was tested over a broad range of concentrations; 60 μg/plate to 6000 μg/plate. Two vehicle controls were plated for all tester strains; both were tested in the presence and absence of the metabolic activator S9. The vehicle controls were 0.001 N hydrochloride acid and deionized water; they were both plated using a 200 μl plating volume.

Appropriate negative and positive controls experiments for each strain were run simultaneously. The diagnostic mutagens were: 2-aminoanthracene at 2.5 μg/plate for TA98, TA100, TA1535, TA1537, and TA1538 with 10% S9 activator; 2-nitrofluorene at μg/plate for TA98, and TA1538 without the S9 metabolic activator; sodium azide at μg/plate for TA100, and TA1535 in the absence of metabolic activation; and ICR-191 at μg/plate for TA1537 in the absence of the 10% S9 activator.
Criteria for a positive response were: for the tester strains TA98 and TA100, a 2-fold increase over the mean revertants per plate of the appropriate vehicle control (i.e., 0.001 N HCl); for tester strains TA1535, TA1537, and TA1538, at least a 3-fold increase over the mean revertants per plate of the vehicle control (i.e., 0.001 N HCl).

Results (Appendix 3) from this Ames Test was reviewed by the Division of Neuropharmacological Drug Products's Pharmacologist Barry N. Rosloff, Ph.D. on August 29, 1994. Both the negative and positive controls induced mutations frequencies as expected. Consistent with the results observed when bupropion (without impurities/degradation products) was used in the Ames Assay, bupropion (with impurities/degradation products) displayed some mutagenic activity in the TA1535 and TA100 strains. In the presence of the S9 activator, bupropion induced a 2.3 fold increase in the mean TA1535 revertants. Positive mutagenic activity was observed in the TA100 tester strain both in the presence and absence of the S9 metabolic activator; a 2.0 fold increase in the mean TA100 revertants was induced by bupropion. Bupropion was not active in the TA98, TA1535, or TA1537 tester strains in either the presence or absence of the S9 metabolic activator.

Cytogenic Analysis of Rat Bone Marrow Treated With Bupropion. Three rat bone marrow cytogenic studies (Reports Nº. TTEP/81/0015, TTEP/82/008, and TTEP/93/058) were performed. These studies have been previously reviewed by the Division of Neuropharmacological Drug Products's Pharmacologist Barry N. Rosloff, Ph.D.; Study Report Nº. TTEP/81/0015 was reviewed on June 15, 1982 (Appendix 1), Study Report Nº. TTEP/82/0008 was reviewed on October 29, 1982 (Appendix 2), and Study Report TTEP/93/058 was reviewed on August 29, 1994 (Appendix 3).

In Study Nº.TTEP/81/0015 five male and five females were gavaged with 0, 100, 200, or 300 mg/kg of bupropion for five consecutive days. For positive control, 5 male and 5 female rats were treated with 0.4 mg/kg triethylenemelamine by intraperitoneal injection for five days. Dr. Rosloff reported that no significant effects were observed in the rats treated with 100 or 200 mg/kg of bupropion. There were increases in all types of chromosomal aberrations tabulated after the administration of 300 mg/kg of bupropion. The positive control triethylenemelamine produced 27.0% aberrants cells.

In Study Nº. TTEP/82/008, rats (12/sex/group; Charles River CD) were treated with a single oral dose of 0, 125, 250, or 500 mg/kg bupropion. The rats were sacrificed either 3, 24, or 48 hours post-treatment. For positive controls, 4 male and 4 female rats were treated with 0.4 mg/kg triethylenemelamine by a single intraperitoneal injection and were sacrificed 24 hours after treatment. Dr. Rosloff reported in his review that bupropion did not produce any chromosomal aberrations.

Study Nº. TTEP/93/0058 (A Cytogenic Study In Rats with Five Daily Doses of 323U66 HCl (Bupropion containing impurities) was conducted in order to determine if several of the impurities present in the sustained release formulation of bupropion could affect its mutagenic potential in rats following five days of oral dosing. The bupropion used in this toxicological study contained 3-chlorobenzoic acid, 268W93, 269W93, 3134W92, 85U77, 20U78, and 827U6 impurities. Five ♂ and five ♀ rats were treated with 100, 200, or 300 (material containing several impurities) or 300 mg/kg of bupropion (material used in clinics) for five consecutive days. Two groups of 5 male and 5 female rats were dosed with five daily oral doses of the vehicle control. All rats were sacrificed 24 hours after the last dosing. The positive and vehicle controls were cyclophosphamide (Cytoxan®). Five male and five female rats received a single intraperitoneal injection of 30 mg/kg or 40 mg/kg of cyclophosphamide; they were sacrificed 24 hours post-treatment.

Results from this study demonstrated that bupropion containing several impurities did not exhibit any significant clastogenic activity. Relative to the vehicle controls, there was no significant increase in the frequencies of structural chromosome aberration. It should be pointed out that results observed with 300 mg/kg differed from the weakly positive response observed in rats treated with 300 mg/kg in Study report TTEP/82/008. Cyclophosphamide, the positive control substance for clastogenic activity, clearly increased the frequencies of structural chromosome aberrations in females treated with 30 and 40 mg/kg and in the 40 mg/kg group for males.

PHARMACOKINETICS

The pharmacokinetics profile of bupropion has been evaluated in dogs, rats, mice , and rabbits following oral and/or i.p. administration. Results from these studies have been reviewed in detail by Barry Rosloff, Ph.D. in HFD 120 and are in Appendix 1.

ABUSE LIABILITY ASSESSMENT

INTRODUCTION

Bupropion, 2-tert-butylamino-3'-chloropropiophenone, is a non-tricyclic antidepressant which structurally resembles amphetamine. In addition to the structural resembles, like cocaine, d-amphetamine and methamphetamine, bupropion prevents the reuptake of dopamine in dopaminergic neurons. Since it is well established that psychostimulants mediate their reinforcing effects by enhancing the activity of the mesolimbic dopaminergic system in the brain (Wise and Rompre, 1989), several behavioral studies have been performed comparing the abuse potential of bupropion with the abuse liability of d-amphetamine, cocaine and methamphetamine.

The drug discrimination paradigm is routinely used in the preclinical assessment of the abuse potential of a drug and it is widely accepted as an animal model for human subjective effects. There is a wealth of preclinical data to support the general statement that for many drugs their subjective effects in humans and their discriminative stimulus properties in animals (eg. LSD-like, amphetamine-like, narcotic-like) parallel one another (Schuster and Balster, 1977; Glennon and Rosecrans, 1981; Chait, *et al.*, 1984; Griffiths *et al.*, 1985). In this paradigm, the animal is required to discriminate between a drug state and a non-drug state. Within the operant chamber, the animal is trained to elicit a response on one lever (eg. right) following drug injection and on the opposite lever (left) following vehicle injection. Once the animal has learned to respond on the correct lever based on the interoceptive cues, novel drugs can be evaluated in order to determine whether or not they elicit similar stimulus properties. Drugs that elicit similar subjective effects in humans are considered likely to produce similar discriminative stimulus effects in animals.

The ability to function as a "positive reinforcer" (i.e., reinforcing efficacy) is another characteristic of all dependence-producing drugs. It is generally accepted in the scientific community that the ability of addictive drugs to serve as "positive reinforcer" is the core property that promotes the development and maintenance of addiction (Thompson and Schuster, 1968; Thompson and Unna, 1977; Balster, 1991). The self-administration paradigm is widely used to determine whether or not a drug can control behavior, that is function as a positive reinforcer and to evaluate the abuse potential of the drug. The self-administration procedures using nonhuman primates and rats have been shown to be a valid and reliable predictor of the potential of a compound to result in drug dependence (i.e., addiction). In this paradigm, the animals are trained to self-administer a known drug of abuse (e.g. cocaine). Once stable responding is maintained, test drugs are substituted for the training drug to determine if they will maintain responding to the new drug. The self-administration paradigm has been valuable in evaluating the reinforcing efficacy of opioids, CNS depressants, cocaine, amphetamine and phencyclidine-like drugs. Preclinical studies have shown that there is a strong concordance between the types of drugs that serve as reinforcers in animals and the many illicit drugs associated with problems of addiction, dependence or abuse by man (Johanson and Balster, 1978; Griffiths *et al.*, 1980; Johanson and Schuster, 1981; Johanson *et al.*, 1987; Woolverton and Nader, 1990).

The dependence-producing potential (i.e., abuse potential) of bupropion has been evaluated in the two standard preclinical models, the drug discrimination paradigm and the self administration model, that are routinely used in the preclinical assessment of the abuse potential of a novel compound. The results of these studies are summarized.

PRECLINICAL STUDIES

DISCRIMINATIVE STIMULUS PROPERTIES. The discriminative stimulus properties of bupropion have been evaluated in several species employing a variety of drug discrimination paradigms. Results from these studies have provided evidences that bupropion is psychoactive -- it can exert discriminative stimulus control over behavior; and that its discriminative stimulus properties (i.e. subjective effects) is similar to the discriminative stimulus effects of cocaine, amphetamine, and caffeine in pigeons, rats and primates (Table 1). Jones *et al.* (1980) first reported bupropion's ability to serve as a discriminative stimulus. Rats were trained to discriminate bupropion (5.0, 10.0, or 20.0, mg/kg, i.p.) from saline in a two-lever drug discrimination paradigm with a FR-10 schedule of food reinforcement. Consistent with what is typically found of other drugs (i.e., cocaine, amphetamine, phencyclidine), the rate of acquisition and degree of discrimination were observed to be training dose-dependent. Learning was faster and discrimination improved in rats trained to discriminate 20 mg/kg of bupropion. Subsequently, the stimulus generalization between bupropion, several tricyclic (imipramine, nortriptyline, amitriptyline, and desipramine), and non-tricyclic (mianserin, viloxazene, and nomifensine) antidepressants, several stimulants (d-amphetamine, cocaine, benzylpiperazine, methylphenidate, caffeine, and phenethylamine) chlordiazepoxide, diazepam, scopolamine, phenobarbital, and morphine were evaluated in rats trained to discriminate 20.0 mg/kg of bupropion. Results from generalization testing suggest that the stimulus cue of bupropion is complex; drugs with stimulant and antidepressant properties generalized to the stimulus cue of bupropion. The stimulants caffeine (12.5 to 50.0 mg/kg, i.p.), d-amphetamine (0.1 to 0.8 mg/kg, i.p.), cocaine (2.5 to 10.0 mg/kg, i.p.), methylphenidate (1.25 to 5.0 mg/kg, i.p.) and benzylpiperazine (1.25 to 10.0 mg/kg, i.p.) generalized to bupropion. The non-tricyclic antidepressants viloxazine (5.0 to 40.0 mg/kg, i.p.), nomifensine (1.25 to 10.0 mg/kg, i.p.), also generalized to the training dose of bupropion in a dose-dependent manner. Bupropion (2.5, 5.0, 10.0, 20.0 and 40.0 mg/kg, i.p.) generalized to the training dose of bupropion in a dependent-dependent fashion. Compounds which failed to generalize to bupropion included: phenethylamine (12.5 - 50.0 mg/kg, i.p.), nortriptyline (5.0 - 20.0 mg/kg, i.p.), amitriptyline (5.0 - 20.0 mg/kg, i.p.), desipramine (5.0 - 20.0 mg/kg, i.p.), minaserin (10.0 - 20.0 mg/kg, i.p.), chlordiazepoxide (2.5 - 10.0 mg/kg, i.p.), diazepam (0.6 - 5.0 mg/kg, i.p.), scopolamine (0.25 - 1.0 mg/kg, i.p.), phenobarbital (5.0 - 20.0 mg/kg, i.p.), and morphine (5.0 - 20.0 mg/kg, i.p.).

Blitzer and Becker (1985) confirmed the findings of Jones and colleagues. Bupropion served as a discriminative stimulus in rats trained to discriminate bupropion (40.0 mg/kg, i.p.) from saline in a two-lever drug discrimination paradigm with a FR-10 schedule of food reinforcement. The psychostimulants cocaine (5.0 - 10.0 mg/kg, i.p.), amphetamine (0.4 - 1.6 mg/kg, i.p.) and the stimulant caffeine (25.0 - 100.0 mg/kg, i.p.) fully generalized to bupropion. Dose-dependent generalization to bupropion was observed with the non-tricyclic antidepressant nomifensine (2.5 - 10.0 mg/kg, i.p.) and the nonstimulant atypical antidepressant viloxazine (10.0 - 40.0 mg/kg, i.p.). The serotoninergic drugs (LSD and quipazine) and the adrenergic compound isoproterenol failed to generalize to bupropion. Dopaminergic (i.e., haloperidol, thioridazine and thiothixene), sertoninergic (cyproheptadine) and adrenergic (propranolol and phenoxybenzamine) antagonists were ineffective in attenuating the discriminative stimulus effects of bupropion.

Kamien and Woolverton (1989) trained rhesus monkeys to discriminate intravenous injections of d-amphetamine (0.67 or 1.33 μmol/kg) from saline in a two-lever operant paradigm under a FR 30 schedule of food presentation. After criterion was established, bupropion (0.25 - 2.0 μmol/kg), amphetamine (0.08 - 2.6 μmol/kg), nisoxetine (1.0 - 1.6 μmol/kg), and cocaine (0.06 - 1.0 μmol/kg) were substituted for d-amphetamine. As seen in the rat studies described above, cocaine, nisoxetine, bupropion, and d-amphetamine substituted for d-amphetamine in a dose-dependent manner.

Similar results were observed in rhesus monkeys trained to discriminate cocaine from saline (Kleven *et al.*, 1990). Kleven *et al.* (1990) evaluated the cocaine-like discriminative stimulus effects of bupropion in rhesus monkeys trained to discriminate cocaine (0.2 or 0.4 mg/kg, i.m.) from saline in a two lever operant task with a FR 30 schedule of food reinforcement. Bupropion (0.1 - 1.6 mg/kg, i.v.) elicited cocaine-appropriate responding in a dose-related manner; complete substitution was observed at the highest dose tested. Other indirect dopamine agonists which generalized to the stimulus cue of cocaine were: GBR 12909 (0.2 - 1.6 mg/kg, i.v.), mazindol (0.025 - 0.4 mg/kg, i.v.), and nomifensine (0.025 - 0.2 mg/kg, i.v.). The ED_{50} for cocaine, mazindol, nomifensine, bupropion, and GBR 12909 was 0.06, 0.07, 0.07, 0.4, and 0.45 mg/kg, respectively. Bupropion and GBR 12909 were approximately 7 to 8 fold less potent than cocaine, nomifensine, and mazindol. With the exception of GBR 12909, the relative potency of these compounds were consistent with their ability to inhibit DA reuptake *in vitro*. In contrast, the norepinephrine reuptake inhibitors tomoxetine (0.8 - 6.4 mg/kg), nisoxetine (0.4 - 1.6 mg/kg), the serotonin re-uptake blocker fluoextine (1.6 - 12.8 mg/kg), the D_1 agonist SKF 38393 (3.2 - 12.8 mg/kg) and the D_2 agonist quinpirole (0.05 - 0.2 mg/kg) failed to generalize to cocaine.

Using a two-lever food reinforced drug discrimination procedure, Lamb and Griffiths (1990) trained rats to discriminate cocaine (23 μmol/kg, i.p.) from saline. Subsequently, generalization tests were conducted with cocaine, nomifensine, diclofensine, imipramine, and bupropion. The discriminative stimulus effects of bupropion were shown to be equivalent to those of cocaine; bupropion (3.2 - 180 μmol/kg) generalized to cocaine in a dose-dependent fashion. Nomifensine (1.0 - 32.0 μmol/kg), cocaine (1.0 - 32.0 μmol/kg), and diclofensine (1.0 - 32.0 μmol/kg) also substituted fully for cocaine in a dose-dependent manner. However, imipramine (1.0 - 56.0 μmol/kg) did not occasion cocaine-appropriate responding.

Consistent with the results reported for rats and primates, bupropion's discriminative stimulus effects appears to have a stimulant-like component (Johanson and Barrett, 1993; Sasaki *et al.*, 1995). Using a fixed-ratio discrimination procedure, Johanson and Barrett (1993) trained eight pigeons to discriminate cocaine (1.0 or 1.7 mg/kg, i.m.) from saline. Subsequently, generalization tests were conducted with cocaine, the psychomotor stimulants d-amphetamine and methamphetamine, the antidepressants l-deprenyl, imipramine, tomoxetine, and bupropion, the dopamine reuptake inhibitor GBR 12909, the 5-HT uptake inhibitors fluoxetine and sertraline, the D_1 agonist SKF 75670, the D_2 agonists quinpirole, the 5-HT_{1A} agonist 8-oh-DPAT and the 5-HT_3 antagonists MDL 72222, LY 278584 and ondansetron. The psychomotor stimulants d-amphetamine, d-methamphetamine completely substituted for cocaine. The anti-depressants l-deprenyl, imipramine, tomoxetine and bupropion also occasioned cocaine-appropriate responding in a dose-dependent manner. Fluoxetine, GBR 12909, quinpirole, SKF 75670 and 8-OH-DPAT partially generalized to the cocaine cue; whereas sertraline and the 5-HT_3 failed to generalize to cocaine.

Sasaki *et al.* (1995) reported on the ability of several compounds, including bupropion, to share discriminative stimulus effects with methamphetamine in pigeons. Four pigeons were trained to discriminate methamphetamine (1.0 or 1.07 mg/kg, i.m.) from saline in a fixed-ratio schedule of reinforcement. The psychostimulants cocaine (0.3 - 3.0 mg/kg, i.nm.), methamphetamine (0.10 - 3.0 mg/kg, i.m.), and amphetamine (0.3 - 3.0 mg/kg, i.m.), the dopamine reuptake inhibitor bupropion (1.0 - 10.0 mg/kg, i.m.), the norepinephrine reuptake inhibitor imipramine (0.3 -3.0 mg/kg, i.m.) and tomoxetine (0.03 - 1.0 mg/kg, i.m.) and the serotonin releaser fenfluramine (3.0 - 10.0 mg/kg, i.m.) substituted for methamphetamine.

REINFORCING EFFICACY. The reinforcing efficacy of bupropion has been evaluated in primates (Bergman *et al.*, 1989; Lamb and Griffiths, 1990). Results from these studies have shown that bupropion does maintain self-administration behavior. Bergman and colleagues (1989) evaluated the positive reinforcing effects of bupropion in five squirrel monkeys trained to self-administer 0.1 mg/kg/infusion cocaine under a second-order FI schedule of reinforcement (FI 10 min [FR 10 or 30:stimulus]) followed by a 1 minute time out. Each session was terminated after the completion of five cycles of the second-order schedule or 90 minutes, whichever occurred first. After stable responding was obtained from session to session, (-)-cocaine (0.01 to 0.56 mg/kg/injection), bupropion (0.1 to 0.3 mg/kg/injection), GBR 12909 (0.03 - 1.0 mg/kg/infusion), mazindol (0.01 - 0.3 mg/kg/injection), and nomifensine (0.01 to 0.3 mg/kg/infusion) were compared for their ability to maintain intravenous self-administration. Bupropion, GBR 12909, methylphenidate, and nomifensine maintained self-administration behavior. The behavioral effects of these compounds were qualitatively similar to cocaine; they maintained response rates and pattern of responding which were the same as those produced by cocaine.

Consistent with the results obtained in squirrel monkeys, bupropion maintained self-administration behavior in baboons trained to perform under a fixed-ratio paradigm. Baboons were trained to self-administer cocaine (0.32 mg/kg/injection) under a FR 80 or 160 schedule of drug delivery. Once stable responding was obtained, nomifensine (0.032 - 0.32 mg/kg/injection), diclofensine (0.10 - 1.0 mg/kg/injection), bupropion (0.10 - 1.0 mg/kg/injection), and imipramine (0.10 - 5.6 mg/kg/infusion) were substituted for approximately 15 days. Bupropion, nomifensine, and diclofensine functioned as positive reinforcers; they all maintained self-administration behavior at levels above those maintained by their respective vehicles. Some doses of bupropion, nomifensine, and diclofensine maintained levels of behavior similar to those maintained under baseline cocaine conditions. In contrast, imipramine did not function as a positive reinforcer.

CLINICAL STUDIES

Results from preclinical abuse liability studies have suggested that bupropion might have amphetamine-like and cocaine-like abuse potential. However, studies in volunteers with a history of amphetamine abuse did not report amphetamine-like activity for bupropion (Miller and Griffith, 1983; Griffith *et al.*, 1983). Griffith *et al.* (1983) compared the effects of bupropion hydrochloride (100, 200, and 400 mg), d-amphetamine sulfate (15 and 30 mg), and placebo in 13 volunteers who had histories of amphetamine abuse. According to a double-blind, randomized crossover design, each dose was given orally at intervals of at least 3 days. The study was conducted on a psychiatric inpatient ward Assessments examined include: Analysis of abuse potential - that is Addiction Research Center Inventory (ARCI) The Single Dose Questionnaire (SDQ), The Amphetamine Self-Rating Scale (ASRS). Physiological Effects: Blood pressure, pulse rate, respiration, body temperature, and pupil diameter. Other measures included: subjective appetit, food intake, and sleep. At doses up to 400 mg, bupropion's pharmacological effects did not resemble those of amphetamine. Bupropion had no effects on the physiologic measures, including blood pressure, pulse rate, body temperature and respiration. Bupropion also did not affect the volunteers appetite, caloric intake or sleep. In the measures of subjective effects, the effects of bupropion were comparable to those of placebo. Bupropion did not elicit amphetamine-like activity on the A (Amphetamine), BG (Benzedrine Group), MBG (Morphine-Benzedrine Group), and the LSD (Lysergic Acid Diethylamide) subscales of the ARCI, and on the SDQ it was perceived as a drug by some of the subjects.

In another clinical abuse liability study, Miller and Griffith (1983) compared the subjective, physiological, and behavioral effects of bupropion (100, 200, and 400 mg) to those of d-amphetamine (15, and 30 mg), and placebo in a randomized double-blind crossover study. In contrast to the other study, the volunteers (n = 14) were polydrug abusers (alcohol, marijuana, amphetamine and cocaine were the primary drugs abused). The study was conducted on the psychiatric inpatient ward. Six treatment sessions, at 72 hours intervals, were approximately 24 hours in duration. All subjects received each treatment condition. Subjective response measures included: ARCI at 0.5, 1, 2, 3, 4, 5, 12, and 24 hours post-treatment, SDQ (cumulative over the first 5 hours). Physiologic measures included: supine systolic and diastolic blood pressure, supine pulse rate, respiration, and oral body temperature at 0, 0.5, 1, 2, 3, 4, 5, 12, and 24 hours following treatment. The behavioral measures include: a measure of appetite consisting of an estimation of hunger. Results from this study suggest that there are clear differences in the subjective profile of bupropion with that of dextroamphetamine. On the ARCI, scales measuring amphetamine-like activity (i.e. Amphetamine Scale and Benzedrine Scale) and the euphoria (Morphine-Benzedrine Scale), dextroamphetamine (15, and 30 mg) had a significantly greater stimulant effect than placebo and bupropion. Some effects were observed following treatment with the high dose of bupropion; bupropion produced a mild elevation over placebo at most time points on the MBG scale. Relative to d-amphetamine, bupropion responses on the SDQ was comparable to placebo. As with placebo, few volunteers identified bupropion as benzedrine (speed). Consistent with its subjective effects, bupropion did not elicit amphetamine-like physiological response. That is, it did not increase blood pressure, pulse rate or respiration.

CONCLUSION.

The published preclinical data has shown that bupropion behavioral profile is similar to that of the psychostimulants and some antidepressants. Drug discrimination studies have suggested that the discriminative stimulus effects ("subjective effects") of bupropion is made up of a stimulant-like and antidepressant-like cues. The stimulants cocaine, d-amphetamine, and caffeine and the atypical antidepressants viloxazine and nomifensine generalized to the stimulus cue of bupropion. The noradrenergic and serotoninergic neuronal systems do not appear to be involved in bupropion's cue; both serotoninergic and noradrenergic agonists were not recognized as bupropion-like in rats. Cross-generalization was also reported. Bupropion substituted for cocaine, d-amphetamine and methamphetamine in rats, and pigeons. Moreover, the reinforcing efficacy of bupropion also appeared to be stimulant-like in primates. Under conditions in which cocaine served as a reinforcer in baboons and squirrel monkeys, bupropion also served as a positive reinforcer.

While the results from preclinical studies have suggested that bupropion may have amphetamine- and cocaine-like abuse potential, amphetamine-like subjective effects were not observed in clinical abuse liability studies; results from clinical abuse liability studies does not indicate that bupropion will have stimulant-like abuse potential. In volunteers with a history of stimulant abuse or polydrug abuse, bupropion at doses that were comparable to those use at therapeutic levels, bupropion was void of amphetamine-like activities in the ARCI and SDQ. Despite these equivocal results, bupropion does appear to have some abuse potential in humans.

Data obtained from Med-Watch has reported some incidences of dependence and withdrawal symptoms associated with bupropion use in the treatment of depression. While these incidences are low relative to the long-term availability of bupropion, the potential for abuse can not be overlooked. One question that surfaces, is will the incidences of dependence and withdrawal symptoms associated with bupropion increase in the proposed new patient population or if more widely available?

LABELING REVIEW.

The proposed draft labeling has been reviewed and the following changes are recommended:

1 Page Deleted

OVERALL SUMMARY.

Zyban® (bupropion hydrochloride) is an antidepressant indicated as an aid to smoking cessation. Zyban® will be available as a sustained release tablet in 3 strengths: 50 mg, 100 mg, and 150 mg. It is recommended that Zyban® be given b.i.d., not to exceed a total dose of 300 mg/day.

Preclinical assessment of the pharmacological profile of bupropion has demonstrated that bupropion's pharmacological profile is similar to that of a CNS stimulant and tricyclic antidepressants. Bupropion increases locomotor activity in mice and rats, induces stereotypic behavior in rats, has an anoretic effect in mice and rats, and elicit amphetamine- and cocaine-like subjective effects in rats and primates. It possesses antidepressant activities in several animal models predictive of antidepressant activities. It was active in the prevention of tetrabenazine-induced sedation in mice and rats; in the reversal of reserpine-induced hypothermia in mice; and the potentiation of behavioral effects of pargyline + DOPA in mice.

Bupropion mediates its antidepressant and stimulatory effects via the dopaminergic system. It is a dopamine uptake inhibitors. Bupropion's ability to suppress withdrawal symptoms and reduce craving may be due its ability to inhibit the reuptake of dopamine in the dopaminergic neurons located in the mesolimbic system.

The acute toxicity of bupropion was assessed in mice and rats. The toxic findings observed the most in both species were of the CNS variety: labored breathing, prostration, ptosis, and ataxia. Compulsive gnawing was also seen frequently in both species. Convulsions were seen frequently in mice.

Results from the oral subacute and chronic toxicity studies performed suggest that bupropion may have hepatotoxic potential. In the 3 month rat study, at all dose levels, there was a low incidence of hyperplasia and "prominent cellular organelles." The liver weight was also increased. Similar results were observed in rats dosed for 3 months with bupropion containing impurities. The liver weight was increased by all doses (75, 150, and 300 mg/kg) evaluated. Minimal centrilobular hepatocellular hypertrophy was observed in the rat dose group. In the rat two year study, there was an increase in incidence of hyperplastic nodules and hepatocellular hypertrophy. In the one year dog study, indicators of liver toxicity included: 1) increased liver weights in all treatment groups.; 2) Histologically the cytoplasm had a fine granular "ground glass" appearance, a dark brown pigment was observed in the hepatocytes and there was some vacuolation of the hepatocytes.; 3) The levels of AP, SGOT, and SGPT were elevated, and there were BSP retention. These were observed at all doses.

The available animal data do not suggest adverse reproductive effects in humans. Bupropion did not affect fertility and reproductive performance in rats at doses up to 300 mg/kg (po). No teratogenic or embryocidal effects were observed in rats at doses up to 450 mg/kg (po) or rabbits at doses up to 150 mg/kg (po). However, bupropion may slightly retard fetal growth since rabbit fetuses exhibited a dose-dependent decrease in fetal weight. The presence of several impurities/degradation products did not alter the reproductive toxicity potential of bupropion in rats; no significant reproductive toxic effects were observed. Hence, the data do not suggest that the sustained release formulation with specified impurities can be a reproductive toxicant in humans.

Bupropion containing impurities/degradation products did not exhibit any significant clastogenic activity in rat bone marrow assay. Bupropion (containing impurities) was weakly positive in bacterial strain TA 100 both in the presence and absence of metabolic activation. Bupropion caused a slight increase in the number of TA 1535 revertants in the presence of the S 9 metabolic activator.

RECOMMENDATIONS.

We have no objections to the approval of this NDA, based on the preclinical pharmacology and toxicology.

The sections of the labeling pertaining to Pharmacology/Toxicology have been discussed with the Sponsor and should be approvable.

BeLinda A. Hayes May 5, 1997

BeLinda A. Hayes, Ph.D., Pharmacologist Date

Concurred by peer reviewer:

Dou Huey (Lucy) Jean May 5, 1997

Dou Huey (Lucy) Jean, Ph.D., Pharmacologist Date

CC: Orig NDA# 20-711
HFD-170/Div File
HFD-170/BHayes
HFD-170/BMcNeil
HFD-170/CWinchell
HFD-340
F/T by: BHayes/4-24-97

APPENDIX 1

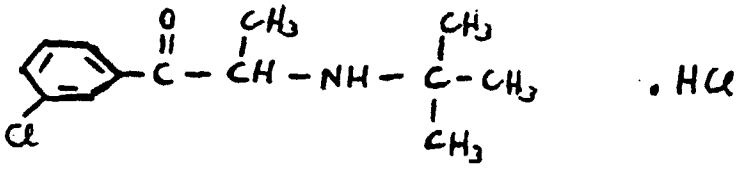
Barry N. Rosloff, Ph.D.
6/15/82

Pharmacologist Review of NDA 18-644
Original Summary

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

DRUG: Wellbutrin Tablets

generic name: bupropion HCl
Code name: BW 323 U (+ others)
chemical name: 2(-tert-Butylamino)-3'-chloropropiophenone HCl (racemic mixture)



CATEGORY: antidepressant

SPONSOR's INDs For BUPROPION:

PRECLINICAL STUDIES REVIEWED:

All studies were performed by sponsor except the following:

6, 7, 8, 10, and 1 of the 2 segment II studies in rabbits:

Ames Test:

Bone Marrow Chromosome Study:

PHARMACODYNAMICS

All doses in mg/kg unless otherwise specified. Abbreviations used:

B	bupropion
AMI	amitriptyline
IMI	imipramine
DMI	desmethylimipramine
A	amphetamine
M	methylphenidate
DA	dopamine
NE	norepinephrine
5-HT	serotonin

A. CNS ACTIVITY

SPECIES	TEST	RESULTS
MOUSE	Prevention of tetrabenazine-induced sedation	ED 50%: B: 12.5 (i.p.) (lowest active dose = 6.5) and 32 (p.o.) AMI: 3.9 (i.p.) A: 1 (i.p.) M: 12 (i.p.)
RAT	Prevention of tetrabenazine-induced sedation	No consistent D-R effect up to 50 i.p.
MOUSE	Reversal of reserpine-induced hypothermia	B active at 12.5 + i.p. AMI - effect at 5 similar to 25-50 of B
RAT	Porsolt behavioral despair model	Approx. ED 50% (3 doses over 24 hr): B: 10 i.p. (inactive at 5) AMI: 12 i.p. IMI: 10 i.p. Effect of B blocked by pre-treatment which depleted brain DA but not by that which depleted brain NE
MOUSE	Potentiation of behavioral effects of pargyline + DOPA	B active at 6.25 + i.p.; IMI equipotent
MOUSE	Locomotor activity	Approx. ED 50%: B: 25 i.p. (increased) (inactive at 6.25) A: 0.5-1 i.p. (increased) M: 6 i.p. (increased) AMI: 12.5 i.p. (decreased)

RAT	Locomotor activity	B: D-R increase at 5-50 i.p.; ED 50% about 5 A: D-R increase at 0.5-4 i.p.; ED 50% about 0.5 IMI: D-R decrease at 10-20; ED 50% about 10 Effects of B and A blocked by pre-treatment which depleted brain DA but not by that which depleted brain NE Effect of B blocked by reserpine (R) and slightly by alpha-methylparatyrosine (AMT); effect of A blocked by AMT only; effect of M blocked by R only
RAT	Operant responding on a FI 60/FI 30 schedule	B increased responding during FI (10-50 i.p.); overall pattern of responding more similar to A than to IMI
RAT	Operant responding on a DLR-20 schedule	B and A decreased rewards at 25-50 and 0.5 - 2 i.p., resp. (B inactive at 5-10). IMI increased rewards at 5-30. Effect of B and A due to increase in early responses.
RAT	Geller conflict test	No antianxiety effects up to 50 i.p.
RAT	Conditioned avoidance responding	Slight non-dose-related increase in avoidance at 10 and 50 (but not at 5 or 25) i.p. for B and at 0.5 - 4 i.p. for A. (IMI - no effect up to 20) Number of intertrial crosses (i.e. locomotor acitivity) increased by B at 10-50 i.p. (No effect at 5) and by A at 0.5 - 2. (IMI - slight decrease at 5 - 20).
RAT	Intracranial self-stimulation	B: dose-related increase at 25-50 i.p. (inactive at 5; slight increase at 10) A: increased at 0.5-4 i.p.; effect similar magnitude to that of B IMI: slight decrease at 10-30 i.p.

RAT	Drug discrimination (Generalization of cues produced by B at 20 i.p.)	Drugs showing generalization: A, cocaine, M, caffeine, phentermine, mazindol, tranylcypromine, viloxazine, nomifensine No generalization: DMI, AMI, TRH, fenfluramine, phenethylamine, scopolamine, diazepam, morphine, pentobarbital, haloperidol, mianserin, trazodone
MONKEY	Self-administration	B produced high rates of self-administration at 1 and 3 per i.v. injection (but not at higher or lower doses); codeine at 0.32 produced lower rates.
RAT	Stereotyped behavior	Increased at 25-100 i.p. (not D-R; no effect at 12.5). Degree considered mild.
MOUSE	Anorexic effect	Active at 50 and 100 (but not at 25) p.o.; fenfluramine at 30 and diethylpropion at 50 had about twice as great effect; also active at 25-50 i.p.
RAT, MOUSE	Brain NE and DA level	No effect up to 100 i.p.
RAT	Brain mitochrondial MAO	No effect up to 10^{-5}M
MOUSE	Brain and liver MAO	No effect in brain up to 100 i.p.; at this dose liver MAO (type A only) inhibited by 25%
RAT	Inhibition of amine uptake into brain slices	IC 50 (μM): NE (hypothalamus) / 5HT (midbrain) / DA (caudate) B: 32 / 90 / 7 AMI: 0.3 / 6.4 / 5.4

RAT	Inhibition of amine uptake into brain synaptosomes	IC 50 (μM):

	NE (hypothal.)	5HT (hypothal.)	DA (striatum)
B	6.5	*	3.4
AMI	0.1	0.35	65
IMI	0.1	0.31	19

*10% inhib. at 10 μM

RAT	NE/DA uptake in vivo (block of AMMT -induced depletion of brain NE/DA)	DA (but not NE) depletion blocked a 50 i.p.; DMI at 30 i.p. slightly blocked NE (but not DA) depletion
RAT	NE/DA uptake in vivo (block of 6-hydroxy-DA-induced depletion of brain NE/DA)	DA depletion blocked at 25-100 i.p. D-R (no effect at 12.5); slight effect on NE at 100. DMI blocked NE (but not DA) depletion at 5-30 i.p.
RAT	ATP - Mg++ - stimulated uptake of NE/DA into brain synaptic vesicles	IC 50 (μM): NE: 33 (5 x less potent than in synaptosomes) DA: 60 (20 x less potent than in synaptosomes)
RAT	Spontaneous release of amines from synaptosomes	No effect on NE, DA, or 5 HT at 10^{-4}M
RAT	DA turnover in striatum (AMT method)	Increased at 50 and 75 (but not 25) i.p.
RAT	Binding assays (brain membrane fragments)	Weak inhibition of binding of WB-41 (IC 50 = 51 μM vs 0.08 for IMI) and clonidine (60% inhibition at 10 μM). Little or no inhibition of the following ligands at B concentrations of 100-1000 μM: dihydroalprenolol, dopamine, spiroperidol, GABA, diazepam, 5-HT
RAT	NE-stimulated adenylate cyclase in cortical slices	No effect at 3000 μM
RAT/MOUSE	Beta receptor desensitization (DHA binding to brain membrane fragments)	Seen with B at 50 i.p. (3x/day for days) in 1/2 of studies; DMI at 10 i.p. (2x/day for 4 days) showed a more consistent effect.

MOUSE	Anticonvulsant	ED 50 = 17 i.p. vs ECS seizures (ED 50 for AMI and DPH = 7 and 9 resp.) ; No effect vs metrazol up to 75 i.p.
RAT	Antagonism of B-induced seizures	Pretreatment with phenobarbital, trimethadione, and chlordiazepoxide (CDP), but not DPH, prevented seizures due to B at 200 i.p.; CDP was most potent (partial protection at 2.5, complete at 5 i.p.) When above drugs given after start of seizures, all (but DPH) shortened the seizure; CDP most potent (activ at 5-10 i.p.)

B. CARDIOVASCULAR/AUTONOMIC

SPECIES	TEST	RESULTS
DOG	Various CV parameters	1) Anesthetized, open chested: at 10-20 i.v., decreases in MAP (20-64% HR (10-20%), CO (15-54%), and right ventricular contractile force (60-70%); effects mostly transient; no effects on above at 5 i.v. 2) Anesthetized, close-chested: at 5-10 i.v., transient decrease in MAP (40-75%) and transient increase in HR (15-34%). When given as a slower infusion (2 mg/kg/min), effects were of smaller magnitude, and in 1 study a slight increase in MAP (5-10%) was seen with 5 mg/kg. No effect on EKG (arrhythmias, PR, QTc) after 5-10 i.v. (2 mg/kg/min). 3) Conscious dogs: After 20 p.o., 10% and 10-20% increased MAP and HR resp., up to 6 hr.; returned to baseline at next measurement (24 hr.)

CAT	B.P. and H.R. in anesthetized cats; vagi sectioned	1, 2.5, 5, and 10 i.v. (4 min. infusions) given sequentially over 2 hr.; non D-R increases in BP (30%) up to 5 but 31% decrease at 10 (all transient); cumulative increase in HR (23%) with some increase after each dose.
RAT	BP and HR in conscious rats	No effect at 25 p.o.; at 50, HR increased (25%) with gradual return to control by 3 hr., no effect on BP
DOG	Respiratory rats (RR) and minute volume (MV) in anesthetized dogs	At 5 i.v., transient increase in RR (68%) and MV (22%); at 10, transient decrease (39%) followed by longer lasting increase (peak 26%) in MV with large increase in RR (100-150%) which was still elevated (60%) at 1 hr.
RAT/GUINEA PIG	Spontaneously beating right atria (in vitro)	1) RAT: D-R decrease in rate and force of contraction at 10^{-6}M +; ED 50% = 1.4×10^{-4}; complete blockade at 3×10^{-4}. AMI and IMI 5-10 x more potent. 2) GUINEA PIG: D-R decrease in rate of contraction at 10^{-5} M +; ED 50% = 1.5×10^{-4}; complete blockade at 3×10^{-4}.
GUINEA PIG	Isolated left atria (stimulated) (in vitro)	No significant effect up to 10^{-4} M
GUINEA PIG	Evoked action potential (AP) in atria (in vitro)	Decreased amplitude of AP and decreased dv/dt, and increased effective refractory period and AP duration at 10^{-5}M + D-R. Excitation totally inhibited at 5×10^{-4}M; IMI and AMI 10 x more potent.
DOG	Isolated Purkinje fibers (in vitro)	Decreased frequency of spontaneous depolarization at 10^{-6} M + ; complete block at $2\text{-}3 \times 10^{-4}$ (AMI, IMI 10 x more potent). At high doses (10^{-4}M +) decreased amplitude of evoked AP, decreased dv/dt, and increased effective refractory period.

DOG	Isolated papillary muscle (<u>in vitro</u>)	At 10^{-4}M +, decreased amplitude of evoked AP and decreased dv/dt and increased effective refractory period. Blocked excitation at 10^{-3}M (AMI and IMI 12-13 x more potent)
CAT	Isolated papillary muscle (<u>in vitro</u>)	No effect on electrically-stimulated contraction up to 3 x 10^{-5}M.
CAT (anesthetized)	1) Contraction of nictitating membrane by preganglionic stim. of cervical sympathetic nerve. (Measure of sympathetic function)	1, 2.5, 5, and 10 i.v. (4 min. infusions) given sequentially over 2 hrs.: 1) No effect on response to stimulation; direct contraction occurred in 2 of 5 cats (slight)
	2) Heart rate after vagal stim. (Measure of parasympathetic function)	2) D-R inhibition; after 10 mg/kg, 50% inhibition with return to baseline after 30-40 min. (In another study, no effect after a single dose of 10).
DOG (anesthetized, vagotomized)	Effect on pressor response to NE and tyramine	D-R potentiation of NE at 0.3 + i.v. (no effect at 0.1); IMI and DMI 10 x more potent and DMI shown to have much longer lasting effect. No consistent effect on tyramine (possible slight inhibition at 9 i.v.) whereas DMI caused 95% inhibition at 1 i.v.
RABBIT	NE uptake into isolated aorta	Weak inhibition; IC 50 = 10 μM (AMI and IMI 50 x more potent)
RAT	Effect on chromodacryorrhea due to methacholine	No effect up to 50 i.p. AMI caused 84% inhibition at 25 i.p. and atropine caused complete block at 1 i.p.
MOUSE	Pupil diameter	D-R dilation at 25-200 i.p. ; AMI and Atropine about 5 and 400 x more potent, resp. B did not impair accomodation as did AMI and atropine
GUINEA PIG	Acetylcholine-induced contraction of ileum	2 x shift-to-right of acetylcholine D-R curve at 10^{-4} M; no effect at 10^{-5}. (IMI caused shift-to-right of 10 x and 490 x at 10^{-6}M and 10^{-5}M, resp., and complete block of acetylcholine at 10^{-4}.)

RAT	Acetylcholine-induced contraction of anococcygeus muscle	No effect at 10^{-5}M
GUINEA PIG	Field-stimulated contraction of ileum (Under conditions said to be a measure of acetylcholine release)	Dose-related inhibition at 10^{-5} M +; EC 50% = 3.1×10^{-5}M.
RAT	NE* release from anococcygeus muscle	No effect at 10^{-4}M. Tyramine increased release 150 and 350% at 10^{-5} and 10^{-4}M, resp.
GUINEA PIG	Chronotropic response of atria to NE and isoproterenol	At 10^{-5}M, NE D-R curve shifted 3 x to left; no effect on response to isoproterenol. At 10^{-4}M, no effect on response to NE in electrically driven atria.
CAT	Response of papillary muscle to isoproterenol	No effect at 3×10^{-5}M
GUINEA PIG	NE-induced relaxation of trachea	No effect on NE response (or spontaneous tone) up to 10^{-4}M
RABBIT	NE-induced contraction of aortic strips	50% inhibition at 10^{-5}M; no effect at 10^{-7}M. No direct agonist action at these concentrations
RAT	NE-induced contraction anococcygeus muscle	Potentiation at 10^{-5}M. (Less than 10 x shift of NE D-R curve to the left.)
RAT	5-HT induced contraction of rat fundus and anococcygeus muscle	No effect on fundus response to 5HT at 10^{-5}M (slight direct contraction by B seen at this concentration); slight (about 10x) shift to right of D-R curve for 5HT on anococcygeus muscle at 10^{-4}M.
GUINEA PIG	5-HT induced contraction of ileum	D-R curve for 5-HT shifted to right at 10^{-5} and 10^{-4}M, dose-related. (approx. 10 x and 100 x shift, resp.)
GUINEA PIG	Histamine-induced contraction of ileum	D-R curve for histamine slightly shifted to the right (less than 10 x) at 10^{-4}M.

C. MISCELLANEOUS

SPECIES	TEST	RESULTS
GUINEA PIG	Local anesthetic activity (cornea)	Active at 2.5-10 mg/ml; equipotent with cocaine.
MOUSE	Effect of grouping on lethality	No effect on i.p. LD 50 for B; LD 5(for A was decreased. B at 25 i.p. decreased the lethality of A at 20 i.p. in grouped mice; AMI at 5 and 10 i.p. did not have this effect.
VARIOUS	Interactions with phenelzine	No significant potentiation of B effects by doses of P causing significant MAO inhibition were seen in several tests (mice-tetrabenazine-induced sedation; rats - Porsolt test, BP, HR; anesthetized dogs - BP, HR; and spontaneously beating or electrically driven rat atria.)
MOUSE	Body temperature (rectal)	1^{o}(c) decrease at 50 i.p. 4^{o} decrease at 100 i.p. (AMI caused 2^{o} decrease at 12.5, A caused 3^{o} increase at 10).

ADME; PHARMACOKINETICS

A. Plasma levels

1. Rat

After 50 mg/kg I.p., plasma levels of unchanged B (method: peaked within 30 minutes and declined with a T 1/2 of 2.3 and 0.95 hr in M and F, respectively. In another study in F, after 30 mg/kg p.o., plasma level of unchanged B (method peaked within 30 minutes and declined biphasically, with an initial (within 1 hr.) sharp decline followed by a terminal T 1/2 of 4 hr.; however plasma levels of metabolites were many times higher than those of unchanged drug (5-30 x up to 8 hr.; 100 x at 12 hr.) and declined much more slowly (T 1/2 about 12 hr.). In another study 10 mg/kg was given p.o. and I.v. Serum (and brain) levels of unchanged B (method: were several fold higher in F than M up to 24 hr. post-dose, although sex differences in T 1/2 were not apparent in this study. Comparison of serum AUC for p.o. and I.v. routes gave a bioavailability of 8 and 21% in M and F, respectively; similar values were found using brain AUC.

In another study, F rats were given 10, 30, or 100 mg/kg orally; plasma levels of unchanged drug increased with increasing doses but slightly less than proportionally at the highest dose; levels of metabolites also increased with increasing dose but less than proportionally at all doses.

2. Mouse

In males, after 50 mg/kg I.p., whole body levels of unchanged drug (method:methyl orange/spectrophotometry) peaked within 15 min. and declined with a T 1/2 of 45 min.; however, metabolite levels remained relatively constant up to 2-4 hr. before declining.

3. Dog

B was given at 10 mg/kg p.c. or I.v. Plasma levels peaked at 1/2 hr. after p.o.; decline in plasma levels by both routes was biphasic with an initial sharp decline within 2 hr. followed by a terminal T 1/2 of about 2 hr. No sex differences in plasma levels were apparent, although N was only 2/sex. A comparison of plasma AUC for I.v. and p.o. gave a bioavailability of 4%.

B. Tissue distribution

Male and female rats were given 50 mg/kg I.p. and sacrificed at 1 hr. Levels of unchanged B (method: were highest in lung (25x plasma levels) followed by kidney, and lowest in plasma. After 50 mg/kg given p.o., highest levels were found in liver

(30-33x plasma levels), followed by lung, and lowest in plasma. (Highest level in males was actually in intestine, but it is not clear how much of this was in intestinal contents.) After both routes, brain levels were 10-12x those in plasma; another study showed that B enters brain rapidly with peak level after p.o. administration reached at the same time as peak plasma level (15 min.). After both routes, levels in all female tissues studied were 2-3x those in male tissues.

In another study, female rats were given labelled B orally at 10, 30, or 100 mg/kg and sacrificed at 1 or 6 hr. Unchanged drug was assayed by RIA and metabolites by subtraction (i.e., total label minus unchanged drug). As found above after p.o., highest levels of unchanged drug were found in liver; lowest in plasma. Tissue levels of unchanged drug increased with increasing dose but not always proportionately (sometimes more, sometimes less). At 6 hr., tissue levels of unchanged drug declined to approximately 1/2 - 1/10 those seen at 1 hr. In contrast to unchanged drug, levels of metabolites were generally highest in lung (3-6x plasma). (Levels in liver were 1.5-2x those in plasma except approximately equal to those in plasma after 100 mg/kg). Tissue levels of metabolites increased with increasing doses but generally less than proportionately. The decline of plasma and tissue levels of metabolites over the 1-6 hr. period was usually much slower than that of unchanged drug; occasionally an increase in metabolite level over this time period was seen. Thus levels of metabolites at 6 hr. were generally several fold higher than those of unchanged drug. (Exception: brain, where metabolite level at 6 hr. was $\leq$ that of unchanged drug). At 1 hr. post-dosing, the level of metabolites in plasma and tissues was also generally greater than that of unchanged drug, but this effect was smaller than that seen at 6 hr. (One exception was brain where levels of unchanged drug were several fold higher than those of metabolites at 1 hr.). In this study pregnant rats also received the same doses of drug on day 16 of gestation, and results were generally similar to those for non-pregnant rats. Fetal levels of unchanged drug were 4-10x those in maternal plasma, and declined in parallel with those in maternal plasma. Fetal levels of metabolites were variable (1/3-3x maternal plasma) with no apparent accumulation during the 1-6 hr. measurement period.

C. Plasma protein binding

(Method:)

SPECIES	CONCENTRATION RANGE (μM)	% Bound
Mouse		75
Rat		77
Dog		81
Man		86
		82
		75

D. Metabolism

B was shown to be rapidly and extensively metabolized in the species studied. One indication of this is the low oral bioavailability of the drug (above) as contrasted with the high degree of absorption of total label after oral dosing (see excretion studies, below), suggesting a large first-pass effect. In female rats receiving 30 mg/kg p.o., plasma levels of metabolites peaked at the same time (0.5 hr.) as those of unchanged drug, and were greater than those of the latter at all time points measured (5-30x up to 8 hr.; 100x at 12 hr.). Tissue levels of metabolites in rats receiving 10, 30, or 100 mg/kg p.o. were also generally greater than those of unchanged drug at 1 and 6 hr. post-dosing (exception: brain). The rate of decline of metabolites from plasma and tissues was significantly slower than that of unchanged drug in rats.

Twenty-four hour urine and fecal samples from rats were treated with 30 mg/kg p.o. showed only trace amounts of unchanged drug (less than 0.2 % of dose, except for 0.3-2.8% in female urine). Acidic metabolites accounted for 86% of the urine label (71% of dose excreted in urine); no basic metabolites were demonstrated. Twenty-four hour urine from a dog given 50 mg/kg p.o. had 88% and 3% of urine label as acidic and basic metabolites respectively (94% of dose excreted in urine). In contrast, in man, 24 hr. urine had 56% and 45% of label as acidic and basic metabolites, respectively (dose not stated for man.)

Two metabolites were identified (method) in 24 hr. rat urines (after 30 mg/kg p.o.): m-chlorohippuric acid (about 25% of dose, or about 1/3 of urine label) and m-chlorobenzoic acid (about 2-3% of dose.) These compounds were also present in 24 hr. feces (2-3% of dose). A conjugate of m-chlorohippuric acid was identified in urine but apparently not quantitated. In 24 hr. dog urine (single dog, 50 mg/kg p.o.) m-chlorohippuric acid and m-chlorobenzoic acid represented about 42% and 4%, respectively, of the dose (45% and 4% respectively, of the urine label).

E. Excretion

1. Rats

30 mg/kg p.o. of labelled B was given; 96 hr. urinary and fecal excretion of label was 78% and 19% respectively, mostly complete by 24 hr. Less than 1% of the dose was found in the carcass at 96 hr. No sex differences were apparent. These results suggest that at least 78% of the drug is absorbed; this figure is probably higher since only trace amounts of unchanged drug was found in feces (above).

2. Dog

One dog received 50 mg/kg p.o. Seven day urinary excretion was 100% of dose (94% in 24 hr.), suggesting complete absorption.

F. Enzyme Induction

1. Plasma and tissue levels of B in rats

Male rats received 0.5, 5, 15, or 50 mg/kg/day, p.o., for 13 days; on day 14 they received 50 mg/kg i.p. and were sacrificed 1 hr later for assay of plasma B. Levels were below acute control (about 50%) at HD only. However, in another study decreases were seen at both 15 and 50 mg/kg (39 and 22% of control level respectively). No effect on liver weight, microsomal protein, or cytochrome P450 was seen. The ability of liver microsomes from these rats to metabolize B and pentobarbital *in vitro* was studied; slight but statistically insignificant increases were seen at the higher doses.

In another study, males and females received 50 mg/kg/day p.o. for 4 days; on day 5 they received 50 mg/kg i.p. and were sacrificed 1 hr. later for assay of tissue B. The levels in the various tissues were about 1/6-1/10 of rats treated acutely.

2. Whole body levels of B in mice

Mice received 50 mg/kg i.p. daily for up to 10 days; whole body B levels were decreased on days 8 and 10 to 63 and 42%, respectively of acute controls. Levels of metabolites were unchanged.

3. Plasma levels of B in dogs

Dogs received 40 or 80 mg/kg/day p.o. for 1 year; on day 366 B levels were 25% and 3-10% of those on day 1 at 40 and 80 mg/kg, respectively. (Levels measured 3 hr. after dosing.)

4. Effect of B on pentobarbital hypnosis (rats, mice)

Doses: 5, 10, 25, 50, 100 or 150 mg/kg/day p.o. for 10 days (latter 2 groups received 50 mg/kg first 5 days). Positive control: phenobarbital at 80 mg/kg. On day 11 animals received a hypnotic dose of pentobarbital.

Results: Rats — Slight decrease (20%) in sleep time at 100-150 mg/kg; 88% decrease with phenobarbital. No effects on lag time to sleep (slight non-dose-related decrease seen with B).

Mice — Dose-related decrease in sleep time; 65% decrease at 150 mg/kg (vs 75% for phenobarbital). No effects on lag time to sleep.

ACUTE TOXICITY:

A. LD 50

SPECIES	STRAIN	ROUTE	SEX	WEIGHT	LD 50 (mg/kg)
Mouse	CD-1	P.O.	M	22 g	544
		P.O.	F	19 g	636
		I.P.	M	27 g	273
Rat	Long-Evans	P.O.	M	109g	607
		P.O.	F	99 g	482
		I.P.	M	130g	263

B. Observed signs

1. Mouse, p.o.

Dosage range = 400-700 mg/kg; deaths seen at all doses and occurred from 5 min.-2 days post-dose. The following signs were seen at all doses: ataxia, prostration, clonic convulsions, ptosis, compulsive gnawing.

2. Mouse, I.p.

Dosage range = 200-300 mg/kg; no deaths occurred at 200 or 250 mg/kg (1/20 deaths at 225). Deaths occurred within 17 min. post-dose. The following signs were generally dose-related and most were seen at all doses: ataxia, clonic convulsions or opisthotonos followed by prostration, labored breathing, decreased respiration, salivation, ptosis, and compulsive gnawing.

3. Rat, p.o.

Dosage range = 400-700 mg/kg; deaths seen at all doses. Most deaths occurred 1-22 hr. post-dose but some were delayed (4-5 days). Most of the following signs were seen at all doses: ataxia, loss of righting reflex, labored breathing, prostration, salivation, ptosis, arched back, and compulsive gnawing.

4. Rat, I.p.

Dosage range = 175-275 mg/kg; no deaths occurred at 175. Deaths occurred within 10-25 min. post dose. Signs were the same as for p.o.; most were seen at all doses.

ACUTE ORAL TOXIC INTERACTION STUDIES (RATS):

A. Bupropion (B) + phenelzine (P)

P, given at highest no-effect and highest non-lethal doses, 3 hr. before B, caused marked decreases in the LD 50 of B. (Degree of decrease hard to calculate from data, but at least 2x). Time to death also decreased. Symptoms potentiated by the combination were salivation, decreased activity, and prostration.

B. Bupropion (B) + 50% ethanol (E)

E given 30 min. after B. The highest non-lethal dose of E decreased the LD 50 of B by 39% in F. The highest non-lethal dose of B caused only very slight decreases in the LD 50 of E (NS in F). No potentiation of toxic signs. (In another study, the highest non-lethal dose of amitriptyline decreased the LD 50 of E by 36% and 20% in M and F, respectively; NS in M).

C. Bupropion (B) + amitriptyline (AMI)

AMI given 3 hr. before B. At 1/2 LD 50 of B + 1/2 LD 50 of AMI, 8/10 and 5/9 deaths were seen in F and M, respectively; thus potentiation occurred in F only. No potentiation of toxic signs.

12 WEEK P.O. TOXICITY IN RATS:

A. Dosage

10 M and 10 F at 0, 150, 300, or 450 mg/kg/day by gavage. (For first 12 days, doses were 100, 200, and 300 mg/kg in LD, MD, and HD, respectively)
Strain: Long Evans

B. Results

1. Observed signs

   a. Urinary incontinence, dose-related
   b. Blood in urine, seen after 5 weeks
   c. Irritability

2. Mortality - 1 LD, 2 HD

3. Body Weight - slightly increased gain in HD F

4. Hematology (post-study)

   a. Hb

   Very slight decrease in all groups, dose-related in F. Maximum mean decrease was to less than 10% below control mean. No extremely low values.

b. Hct

Slight decrease in all M groups, not dose related. Mean values 10% below control. No extremely low values. No effect in F.

c. MCHC

Very slight dose-related decrease in all F groups; no unusually low values.

5. Blood chemistry (post-study)

a. glucose

Slightly decreased at MD and HD (about 15% below C); no unusually low values

b. total protein

Dose-related increase in all groups. Mean at HD 9 and 16% above control in M and F, respectively. Most rats were affected but none showed extremely large elevations.

c. Other parameters measured: BUN, SGOT, SGPT, AP

6. Organ weights

a. Liver

Increased absolute and relative weight at MD and HD. At HD, mean relative weight was 48 and 35% above control in M and F, respectively.

b. Kidney

Slight dose-related increase in absolute and relative weight in all groups but LD M. At HD, mean relative weight was 12 and 14% above control in M and F, respectively.

c. Other organs weighed: heart, spleen, testes, brain

7. Gross pathology - no drug effect

8. Histopathology - (H+E stain)

Hyperplasia and/or prominent cellular organelles seen in liver of 0/20 controls, 3/20 LD, 4/20 MD, and 4/20 HD.

55 WEEK P.O. TOXICITY IN RAT:

A. Dosage

60 M + 60 F at 0 (untreated), 0 (vehicle), 25, 50, or 100 mg/kg/day, by intubation. Charles River CD rats used.

B. Results

1. Observed signs

a. Most prevalent: dose-related increase in yellow staining of hair around anogenital region

b. Also seen mainly in treated rats: dry brown material around nose or mouth and moisture around mouth.

2. Mortality

No drug effect. Overall mortality 50-60% in M and 20-25% in F. Most deaths said to be due to intercurrent respiratory disease. Untreated controls had lower mortality than other groups.

3. Bodyweight gain

Very slight decrease in HD M; final weight 5% below vehicle control.

4. Food consumption - no drug effect

5. Ophthalmoscopy

(pre-study and at 12 months in all rats, binocular indirect ophthalmoscope)

No clear drug effect. Cataracts in 2 LD and 2 MD; retinal detachment in 1 LD; neither of these findings at HD

6. Laboratory tests - (10/sex/group at 12 months)

a. Hematology

1. 1 HD M and and 1 HD F had low RBC, Hb, and Hct, and (in F only) high WBC. Group means showed no effect.

2. Other parameters measured: WBC differential.

b. Blood chemistry

1. Glucose

Slight decrease seen in most HD rats (mean 15% below control); slight increases seen in most MD F (mean 18% above control). Values stated to be WNL.

2. BUN

Elevated in 1 MD and 2 HD F (2x control). One HD F had marked focal chronic nephritis; no unusual kidney histopathology in the MD; not examined in the other HD.

3. SGOT/SGPT

Elevations of both seen in 1 LD F and 1 MD M. The M also had slightly decreased albumin (no change in total protein, slightly increased RBC, Hb, and Hct, decreased WBC, slightly increased % neutrophils, and slightly decreased % lymphocytes. No unusual histopathology in the M (kidney and spleen only); not performed in the F.

4. Total protein

Very slight increase in 1 MD F and 1 HD F; moderate increase (50% above control) in 1 HD F (the same rat with elevated BUN and nephritis, above)

5. Other parameters: AP, albumin

c. Urinalysis

1. Color mostly "straw" in control M and F and LD M, and mostly "light straw" in all other groups

2. Other parameters: volume, pH, SG, albumin, glucose, bilirubin, occult blood, ketone, sediment.

7. Organ weight

a. Liver

Absolute weight increased in all groups; dose-related increase in relative weight in all groups (15-18% above control at HD)

b. Kidney

Absolute and relative weight increased in all groups except LD M; effect on relative weight generally dose related, 16 and 7% above C in HD M and HD F, respectively.

c. Thyroid/parathyroid

Increased absolute and relative weight in HD M and HD F (relative weight 57 and 22% above C, respectively)

d. Adrenal

Increased absolute and relative weight in HD M (relative weight 29% above C)

e. Pituitary

Increased absolute and relative weight in HD M when compared to untreated control (relative weight 36% above untreated control); vehicle control value elevated due to one extremely high value.

f. Other organs weighed: testes, brain

8. Gross pathology

No clear drug effect. Enlarged liver seen in 1/29 MD M, 1/24 HD M, and 1/18 HD F which died before termination (vs 0/30 control M and 0/14 control F)

9. Histopathology

(Routine H and E exam in 15/sex in C and HD; spleen and kidney only in 15/sex in LD and MD. Iron stained sections of liver, kidney and spleen in 15/sex/group and bone marrow in 15/sex in C and HD were examined. All rats examined in C and HD were sacrificed at termination, as were 18/30 LD and 23/30 MD, the remainder having died after week 30 except 2 MD M (weeks 20 and 25).

a. Spleen

By H & E stain, increased amount of hemosiderin pigment in red pulp seen in all M groups regarding both incidence and severity and in all F groups regarding severity only (incidence in control F nearly 100%):

		M	F
Incidence:	C	7/15	14/15
	LD	10/15	15/15
	MD	12/15	14/15
	HD	15/15	13/15
Severity: (Sum of severity scores/# of rats affected	C	2.7	3.5
	LD	2.8	3.8
	MD	2.9	3.9
	HD	3.4	4.2

However, by iron staining, hemosiderosis was present in all rats examined with the only pronounced effect on severity seen in HD M with smaller equivocal effects in LD and MD M and HD F:

	M	F
C	3.5	4.6
LD	3.9	4.4
MD	3.8	4.5
HD	4.3	4.8

b. Kidney

1. Increased incidence of yellowish-brown pigment in cytoplasm of proximal convoluted tubule at HD (24/30 vs 9/30 in control); severity also greater.

2. Slightly increased incidence of focal chronic nephritis at HD (14/30 vs 9/30 control); severity slightly increased.

3. Slightly increased severity of hemosiderosis (iron stain) at HD; no effect on incidence (seen in nearly all rats.)

c. Lung

Focal aggregates of alveolar macrophages with hemosiderin pigment seen in all C and HD, but severity slightly greater at HD

d. Liver

Increased incidence of hemosiderosis (iron stain) in HD M (11/15 vs 5/15 control) but no effect on severity.

23-24 MONTH RAT CARCINOGENESIS STUDY

A. Dosage

75 M and 75 F at 0, 100, 200, or 300 mg/kg/day, by gavage. (HD received 500 mg/kg on days 1-15 and 400 mg/kg days 16-74)

Interim sacrifice (5/sex/group) at 6 months; terminal sacrifice at 23 and 24 months in M and F, respectively

Strain: Charles River CD

B. Results

1. Observed signs

   a. Intermittent convulsions, dose-related (10-20% incidence at HD for the selected weeks shown)

   b. Moisture and red/brown material around mouth, dose-related.

   c. Yellow material on anogenital region, dose-related.

2. Mortality

   Dose-related increase in all groups except LD M. One-hundred week survival:

	M	(Week of 50% Survival)	F	(Week of 50% Survival)
C	27/70	(81)	49/70	(> 104)
LD	32/70	(95)	40/70	(> 104)
MD	20/70	(81)	25/70	(90)
HD	3/70	(42)	14/70	(51)

3. Bodyweight

   a. M - decreased gain in all groups (final weight 10, 17, and 16% below control in LD, MD, and HD, respectively.)

   b. F - slight dose-related increased weights seen during most of study (10% above C at HD), although final weights 2-4% below C.

4. Food consumption - very slight increase at HD

5. Ophthalmoscopic exam

   (All rats, pre-drug and months 6, 12, 18, 24; binocular indirect ophthalmoscope)

Slight increase in incidence of cataracts and/or lens opacity at MD. (Not seen at HD; however this might have been obscured by the high mortality at HD and the fact that most of the findings occurred late in the study.)

6. Laboratory tests

(10/sex/group at months 6, 12, and 23/24 [except only 3 M left at 23 months]).

a. Hematology

No consistent drug effects (RBC, Hct, Hb, WBC, differential)

b. Blood chemistry

1. Glucose - slight decrease in all M groups at months 6 and 23 (but not 12) and in HD F at 24 months

2. Other parameters: BUN, total protein, albumin, AP, SGOT, SGPT

c. Urinalysis

1. Volume - slight dose-related increase in all M groups at 23 months

2. Other parameters: color, appearance, pH, SG, albumin, glucose,, bilirubin, occult blood, ketones, sediment.

7. Organ weight

(5/sex/group at 6 months, plus all surviving animals at termination)

a. Liver

Dose-related increase in absolute and relative weight in all groups. Relative weight at HD 32% and 51% above control at 6 months and termination, respectively.

b. Kidney

1. M - dose-related increased in absolute and relative weight in all groups. Relative weight at HD 21% and 45% above control at 6 months and termination, respectively.

2. F- increased absolute and relative weight at HD at 6 months (relative weight 13% above C) and in all groups at termination (not dose-related; relative weight 16% above C)

8. Gross pathology

(Separate incidence values were presented for deaths at 0-6 months, 6 month interim sacrifice, deaths from 6 months - termination, and terminal sacrifices.)

a. No drug effects at 6 month sacrifice; no effect among deaths 0-6 months aside from increased incidence of bloody oral or nasal discharge at MD and HD (see observed signs).

b. Urine/feces/red material on ventral surface or tail increased in all F groups (see observed signs).

c. Liver

1. Mass/nodule/raised area - slight increase in F at termination (3/43, 8/38, 2/24, 5/12 in C, LD, MD, HD, respectively) but no effect among deaths (6 months-termination) or overall combined.

2. Dark red/brown foci/hemorrhagic foci/area - increase in all groups but LD F at termination:
M: 4/27, 17/31, 11/20, 1/3 in C, LD, MD, HD
F: 7/43, 6/38, 10/24, 3/12 in C, LD,, MD, HD

3. Grey/yellow foci/area - slight increase in F at termination (1/43, 5/38, 2/24, 3/12 in C, LD, MD, HD)

d. Lung

Increased incidence of yellow/white/grey foci in all groups at termination:
M: 9/27, 12/31, 12/20, 3/3
F: 9/43, 17/38, 19/24, 11/12

9. Histopathology

(H&E stain. Complete exam in C, MD, and HD rats which died during the study [except not done in MD which died during first 6 months], in 5/sex in C and HD sacrificed at 6 months, and in all C, MD, and HD which were sacrificed at termination. Exam at LD limited to liver, tumors, and gross lesions suspected of being tumors.)

(Separate incidence values for non-neoplastic findings were presented for deaths at 0-6 months, 6 month interim sacrifice, deaths from 6 months-termination, and terminal sacrifice. One exception to this was liver findings which were presented in 1 overall table; however certain findings were tabulated separately by M.I.T. consultants and will be presented below. Neoplastic findings were also presented in one overall table.)

a. Neoplastic findings

There were no drug-related increases in either total benign or malignant tumors (corrected for increased mortality in drug groups by use of life-table analyses) or in any specific tumor type. Tumors with the highest incidence were mammary fibroadenoma/adenoma in F and pituitary adenoma; all others generally had an incidence of less than 5%. (Results for hyperplastic nodules in liver, which were increased in drug groups are given below under "non-neoplastic" findings since there is no evidence that these nodules were neoplastic. (See Evaluation.)

b. Non-neoplastic findings

1. Liver

a. Hyperplastic nodules

Increased incidence in all groups. The following incidence values are taken from an M.I.T. consultant report which breaks down the results into incidence at terminal sacrifice vs. incidence among rats which died week 28 and later:

		M	(%)	F	(%)
Terminal Sac:	C	2/27	(7%)	1/43	(2%)
	LD	7/31	(23%)	7/38	(18%)
	MD	7/20	(35%)	5/24	(15%)
	HD	1/3	(33%)	4/11	(36%)
Deaths:	C	0/37	(0)	0/25	(0)
	LD	1/29	(3%)	2/23	(9%)
	MD	0/43	(0)	3/40	(8%)
	HD	0/47	(0)	1/35	(3%)

It can be seen that the majority of nodules were not observed until the terminal sacrifice, suggesting a late appearance.

b. Hepatocellular hyperplasia

Increased at LD and MD. Described mainly as focal and slight or very slight:

M: 10/73, 26/72, 15/70, 2/64 in C, LD, MD, HD
F: 16/74, 29/68, 23/71, 5/59 in C, LD, MD, HD

c. Hepatocellular hypertrophy

Increased in all groups. Described mainly as focal or multifocal (with some diffuse in MD and HD F), and slight or very slight:

M: 1/73, 6/72, 15/70, 4/64 in C, LD, MD, HD
F: 3/74, 13/68, 31/71, 31/59 in C, LD, MD, HD

2. Lungs

a. Dark brown pigment/macrophage accumulation, alveoli

Increased at MD and HD (both sexes) among deaths (6 months-termination) and at termination. Combined incidence = 32/133, 0/2, 66/129, 46/103 in C, LD, MD, HD

b. Interstitial inflammatory/lymphocytic/mononuclear infiltrate

Increased in MD and HD F among deaths (6 months-termination) and at termination. Combined incidence = 15/68, 0/2, 36/65, 18/50 in C, LD, MD, HD.

c. Congestion/edema

Increased incidence at HD among deaths (0 - 6 months) but equivocally among deaths (6 months - termination)

3. Spleen

Hemosiderosis - Increased at MD and HD among deaths (6 months-termination) and (in F only) at termination. Combined incidence:

M: 2/64, 0/2, 15/63, 5/49 in C, LD, MD, HD
F: 4/68, 0/1, 18/64, 14/57 in C, LD, MD, HD

4. Cervical lymph node

Slightly increased incidence of dark brown pigment laden macrophage accumulation at MD and HD among deaths (6 months-termination) and (in F only) at termination. Combined incidence:

M: 3/59, 0/0, 10/53, 5/46 in C, LD, MD, HD
F: 7/65, 0/0, 8/52, 6/45 (deaths only: 1/24, 0/0, 5/32, 5/34) in C, LD, MD, HD

5. Mesenteric lymph node

Increased incidence of pigment laden macrophage accumulation/hemosiderosis at MD and HD among deaths (6 months-termination):

M: 1/35, 0/0, 9/43, 7/44 in C, LD, MD, HD
F: 1/24, 0/1, 7/39, 7/33 in C, LD, MD, HD

An equivocal increase also seen at HD at termination

[illegible]

Increased incidence of light cell proliferation among deaths (6 months-termination) at HD:

M: 0/37, 0/0, 2/40, 9/46 in C, LD, MD, HD
F: 0/25, 0/0, 0/30, 4/34 in C, LD, MD, HD

[illegible]Kidney

Slightly increased incidence of chronic nephritis at MD and HD among deaths (6 months-termination) and (in F only) at termination.

M (deaths only):	12/37, 0/0, 27/44, 24/48
F (deaths & termination):	17/67, 0/0, 26/64, 20/47

21-22 MONTH MOUSE CARCINOGENESIS STUDY

A. Dosage

100 M + 100 F at 0, 50, 100, or 150 mg/kg/day, by intubation.

(MD and HD received 50 mg/kg for first 2 weeks; HD received 100 mg/kg for next 4 weeks.)

[illegible] at 21 and 22 months in M and F, respectively.

Strain: Charles River CD-1

B. Results

1. Observed signs

a. Clonic convulsions at MD and HD, dose-related, throughout the study. At week 92, 11% of HD had convulsions within 3 minutes after dosing.

b. Increased incidence of moist red substance on urogenital region in all F groups. At week 96: 11, 23, 39, and 50% in C, LD, MD, and HD respectively. (Due to uterine bleeding - see pathology results.)

2. Mortality

Increased in all M groups, partly dose-related, and slightly at HD

a. Survival at termination:

	M	(WEEK OF 50% SURVIVAL)	F	(WEEK OF 50% SURVIVAL)
C	49/99	(91)	28/100	(90)
LD	30/98	(81)	22/100	(89)
MD	33/100	(75)	31/99	(83)
HD	19/100	(66)	20/100	(81)

3. Bodyweight - no drug effect

4. Food consumption - no drug effect

5. Gross pathology

a. Uterus

1. Increased incidence of nodules/masses to about the same extent in all drug groups; seen both at terminal sacrifice and among deaths. Overall incidence = 15/100, 42/100, 41/100, and 41/100 in C, LD, MD, and HD, respectively. According to the text, these masses/nodules were actually extremely dilated veins with thrombosis. (See histopathology, below.)

2. Increased incidence of dark red-grey areas, "highly vascularized/hemorrhagic/red contents," and thickening of uterine wall in all drug groups. The incidence of these findings was generally under 10% and not [illegible] except for dark red-grey areas.

b. Spleen

Enlarged spleen seen in all F groups, partly dose-related, both at termination and among deaths. Overall incidence: 18/100, 39/100, 37/100, and 48/100 in C, LD, MD, and HD, respectively.

Stomach

Slight increase in red foci/hemorrhagic/bloody contents in all F groups both at termination and among deaths. Overall incidence: 2/100, 5/100, 15/100, 13/100 in C, LD, MD, and HD, respectively.

Intestines

Slight increase in hemorrhages/blood/reddish contents in HD M and HD F among deaths: 1/55, 1/75, 6/81, 7/81 in CM, CF, HD M and HD F, respectively.

c. Histopathology

(H + E stain. Routine exam in all C and HD; spleen, uterus, and gross lesions considered possibly neoplastic in LD and MD.)

a. Neoplastic findings

There were no drug-related increases in either total benign or malignant tumors (corrected for increased mortality in drug groups by use of life-table analyses) or in any specific tumor type. The overall tumor rate was low, the highest being for lung adenoma (17% in control, 8% at HD); incidence of all other tumor types was less than 5%.

b. Non-neoplastic findings

1. Uterus

a. Increased incidence of extremely dilated blood vessels, with thrombosis, in all F groups, dose-related, both at termination and among deaths. Overall incidence: 15/100, 37/100, 52/97, and 62/98 in C, LD, MD, and HD, respectively.

b. Increased acute metritis/pyometritis in all F groups, dose-related: 4/100, 12/99, 17/98, and 21/98 in C, LD, MD, and HD, respectively.

c. Slight increase in hemorrhage in all F groups: 1/100, 3/100, 8/97, and 5/98 in C, LD, MD, HD.

2. Spleen

a. Increased hematopoietic activity in all F groups, partly dose-related, among deaths but not at termination. Overall incidence: 17/100, 35/100, 53/99, 52/98 in C, LD, MD, HD.

b. Slight increase in incidence of foamy macrophages in all groups: 0/200, 6/198, 6/199, 7/197 in C, LD, MD, HD.

3. Lymph nodes

Slightly increased incidence of foamy macrophages with cellular debris in HD M and HD F among deaths: 0/45, 0/71, 4/80, and 7/79 in CM, CF, HD M, and HD F, respectively.

4. Liver

a. Slightly increased extramedullary hematopoiesis in HD F, among deaths: 14/79 vs 4/75 in control.

b. Slightly increased incidence of lymphocytic infiltrate in HD F, among deaths: 19/79 vs 6/75 in control.

5. Heart

Increased incidence of thrombus in HD M, among deaths: 7/80 vs 1/55 in control.

6. Lung

Increased incidence of congestion/hemorrhage in HD M both at termination and among deaths. Overall incidence was 34/100 vs 14/99 in control.

7. Kidney

Increased incidence of brown pigment in tubule epithelium in HD F, among deaths: 11/79 vs 1/75 in control.

8. Testes

a. Increased incidence of atrophic tubules in HD M both at termination and among deaths; overall incidence = 21/99 vs 6/100 in control. No effect on incidence of aspermatogenesis.

b. Increased incidence of amyloidosis in HD M, among deaths: 31/80 vs 5/55 in control.

9. Stomach, small intestine

Increased incidence of ulcer, congestion, hemorrhage, and inflammation at HD, related to gross findings, above. Very low incidence.

90 DAY P.O. TOXICITY IN DOGS

A. Dosage

2M + 2F at 0, 15, 35, or 75→150 mg/kg/day, in capsules. (Dosage increase at HD occurred on day 45.)

Strain: beagle

B. Results

1. Observed signs

No effect. (Preliminary study showed convulsions and death with acute oral doses of 150-400 mg/kg.)

2. Mortality - none

3. Bodyweight - no effect (no data shown)

4. Hematology

(pre-drug and days 45 and 90)

a. Hb

Slight decrease in all groups. Not related to dose or time except greatest effect in HD at 90 days (28% below control). No extremely low values.

b. Hct

Same as above. Mean in HD at 90 days 21% below control.

c. Other parameters measured: MCHC, platelets, WBC, differential, RBC appearance, clotting time, sedimentation rate, icterus index, osmotic fragility, prothrombin time.

5. Blood chemistry

(pre-drug and days 45 and 90)

No drug effects. One HD F had slightly elevated BUN and creatinine but no associated histopathology. Other parameters measured: glucose, cholesterol, thymol turbidity, AP, SGOT, SGPT, total protein, methemoglobin, Na, K, BSP retention, bilirubin.

6. Urinalysis

(pre-drug and days 45 and 90)

No drug effects (volume, color, appearance, SG, pH, bilirubin, urobilinogen, albumin, occult blood, sugar, acetone bodies, sediment exam).

7. Fecal exam

(pre-drug and days 45 and 90)

No drug effects (color, appearance, occult blood)

8. Organ weights

a. Liver

Slight increases in absolute and relative weight at HD (Relative weight 18% above control)

b. Other organs weighed: heart, spleen, adrenals, kidney, brain, testes, thyroid

9. Gross pathology - no drug effects

10. Histopathology (H&E stain) - no drug effects

52 WEEK ORAL TOXICITY IN BEAGLE DOGS:

A. Dosage

8M + 8F at 0, 40, 80 or 80 → 150 mg/kg/day, in gelatin capsules. Dosage escalation at HD complete by 5 weeks.

At 6 months, 2M + 2F from controls, LD, and MD, and 2M + 1F from HD were sacrificed and necropsied. At 12 months, 4M + 4F per group were sacrificed. All remaining dogs were kept for 2 month withdrawal period, then sacrificed.

B. Results

1. Observed signs

LD - none

MD - ptyalism, emesis, and dry nose and/or mouth noted occasionally

HD

a. Weakness in 4 dogs, mainly first 3 months.

b. Emesis, ptyalism, and dry nose and/or mouth noted occasionally to several times per week.

c. General body trembling noted occasionally in a few dogs.

d. Clonic convulsions in 1 F, week 35. Dosing stopped for 3 weeks; upon re-initiation of dosing, frequent emesis and evidence of convulsions were noted, and death occurred after 4 days.

e. One F died week 17 preceded by body trembling and blood in refuse pan.

2. Mortality

a. 1 MD M, week 35 (No signs prior to death)

b. 1 HD F, week 40 (Signs given above, 1 d)

c. 1 HD F, week 17 (Signs given above, 1 e)

3. Bodyweight gain

Decreased at HD (Overall weight gain = 27% (M) and 19% (F) for controls, and 6% (M) and 7% (F) for HD. During withdrawal period, HD gained more than controls.)

4. Food consumption

   No treatment effect except very slight decrease in HD M.

5. Water intake/urine output (measured pre-drug, and months 3, 6, and 12, over 5 consecutive days, in controls and HD).

   No treatment effects

6. Ophthalmoscopic exam (performed on all dogs pre-drug and months 3, 6, and 12, using binocular indirect ophthalmoscope)

   No treatment effects

7. ECG (measured in all dogs pre-drug and months 3, 6, and 12.)

   ECG tracings for all dogs were included in the appendix. There were apparently no calculations of segment lengths performed. The report states there were no treatment effects.

8. Hematology (measured pre-drug and months 3, 6, and 12; after withdrawal period, only total RBC, Hct,and Hb measured)

   a. Total RBC

      1. Moderate decrease in 1 LD dog, month 6

      2. Slight decrease at MD, month 6

      3. Slight decrease at HD, months 3, 6, and 12, but least effect month 12.

   b. Hct

      1. Moderate decrease in 1 LD dog, month 6

      2. Slight decrease at HD, months 3, 6, and 12, but least effect month 12.

   c. Hb

      1. Moderate decrease in 1 LD dog, month 6

      2. Very slight decrease at MD, month 6

      3. Slight decrease at HD, months 3, 6, and 12, but least effect month 12.

d. The above effects on RBC, Hct, and Hb were greatest at HD, but did not appear to be progressive over time. The mean decreases at HD were less at 12 months vs 6 months. Some individual dogs with low values at early times showed normalization at 12 months. The values in treated dogs after the withdrawal period were comparable to controls.

e. Total WBC - Increased in 2 HD dogs, month 12

f. Differential WBC

Slight increase in neutrophils and slight decrease in lymphocytes at LD, months 6 and 12, and MD, month 12.

g. No treatment effects on platelet count

9. Blood chemistry (measured pre-drug and months 3, 6,and 12; after withdrawal period, only cholesterol, AP, SGOT, and SGPT were measured)

a. AP (alkaline phosphatase)

Dose-related increase seen at all months. The magnitude of the increase, relative to controls, increased over time. At 12 months, group mean values were approximately 2x, 2x, and 4x that of the controls in LD, MD, and HD, respectively. Most of the treated dogs had elevations. However, none of the recovery animals had elevations after the 2 month withdrawal period.

b. SGPT

1. 3 months - slight elevation in 1 LD and 1 MD; slight to moderate elevation in most HD.

2. 6 months - slight elevation in 1 control, 1 LD, 1 MD, and most HD.

3. 12 months - slight elevations in most MD; slight to moderate elevations in all HD.

4. The magnitude of the elevation was greatest at HD but not clearly related to duration of treatment: The group mean values at HD were approximately 4x, 1.7x, and 3.6x that of controls at 3, 6, and 12 months, respectively. At MD, the largest increase (1.6x control mean) was at month 12. After the withdrawal period, slight elevations were seen in 2 of the 3 dogs at MD and HD (group mean values approximately 1.5x control mean); the elevations in the 2 HD dogs were considerably less than those seen at 12 months.

c. SGOT

1. 3 months - moderate elevation in 1 LD; slight elevations in 2 LD, 1 MD, and 6 HD.

2. 6 months - moderate elevation persisted in 1 LD; slight elevations in 1 control and 1 MD.

3. 12 months - slight elevation in 1 MD and most HD (group mean at HD approximately 2x control mean). The elevations in individual MD dogs at 12 months were not significantly different in magnitude than those seen at 3 months.

4. No treatment-related effects seen after withdrawal period.

d. BSP retention

Slight increases in approximately 1/2 HD at month 3, and in most HD at month 12 (mean value approximately 2x control mean). Very slight increase in mean value seen at MD at month 12.

e. Total protein

Slight decrease in most HD dogs at 6 and 12 months

f. Albumin

Very slight decrease in most HD dogs at 6 and 12 months

g. No consistent treatment effects on cholesterol, glucose, BUN, A/G ratio, prothrombin time, bilirubin, Na, K, or creatinine.

10. Urinalysis (measured pre-drug and months 3, 6, and 12)

a. Slight, dose-related decrease in pH in all treatment groups at all months.

b. No consistent treatment effects on urine volume, urine color and appearance, specific gravity, albumin, glucose, bilirubin, occult blood, or sediment.

11. Organ weights

a. Liver - increased absolute and relative weights in all groups at both 6 and 12 months, dose-related. No treatment effects in recovery dogs.

b. Kidney - increased absolute and relative weights in all groups, dose-related, at 6 months; at 12 months increase in relative weight only, at MD and HD only. After recovery period, no treatment effect on absolute weight; relative weight increased at LD and HD.

c. Ovary - absolute and relative weight increased at HD at 6 months, but decreased at 12 months. No effect at HD after recovery, except slightly increased relative weight due to decreased body weight.

d. Thyroid/parathyroid - increased absolute and relative weight at HD at 6 months only.

12. Gross pathology

a. 6 month interim sacrifice

1. Slightly yellowish liver in 1 LD

2. In the HD F which died: "Pulmonary edema and congestion and slight hydrothorax in this dog were probably compound related, agonal changes."

b. Terminal sacrifice

1. No treatment-related effects in dogs which were sacrificed at termination or after the withdrawal period.

2. In the MD M which died: hemorrhages in tracheal and bronchial mucosa and scattered hemorrhages in lung

3. In the HD F which died: hemorrhages in mucosa of stomach and small intestine, and congestion and/or hemorrhages in several other organs.

13. Microscopic pathology (H and E was only stain used, except for Giemsa-Wrights for bone marrow)

a. Liver

1. At the terminal sacrifice, the hepatocytic cytoplasm was described as having a finely granular "ground glass" appearance in 7/8 MD and 8/8 HD. (0/8 in controls and LD). The severity of this was described as slight to moderate, and was not related to dose. This finding was not reported among dogs sacrificed at 6 months, or after the recovery period. It was not reported for the 3 dogs which died during the study.

2. At 6 months, greenish-brown pigment in Kupffer cells seen in 0/4 controls, 2/4 LD, 1/4 MD, and 1/4 HD (severity was very slight to slight, not dose-related.) At termination, brown pigment in Kupffer cells was seen in 1/8 controls, 0/8 LD, 2/8 MD, and 1/8 HD; however, dark brown pigment (very slight to slight) located in "phagocytic cells, portal areas" was seen in 4/8 HD but not in other groups. In addition, at termination, fine dark brown pigment in hepatocytes was seen at 4/8 MD (very slight to slight) and 2/8 HD (slight), and in 0/8 in controls or LD. No findings of liver pigment were seen in the 3 dogs which died during the study. Among the recovery animals, dark brown pigment in Kupffer cells (very slight) was seen in 1/4 controls, 1/4 LD, 0/3 MD, and 1/3 HD, and fine brown pigment in hepatocytes was seen in 0/4 controls, 1/4 LD, 1/3 MD, and 3/3 HD (severity very slight to slight, not dose-related).

3. Slight coarse vacuolation of periportal hepatocytes was seen in 3/8 HD at terminal sacrifice. Hepatocytic vacuolation was also seen in the MD dog which died during the study. At the 6 month sacrifice, centrolobular hepatocytic vacuolation was found in 1/4 LD (slight) and 1/4 MD (moderate), but not found in the 4 control or HD dogs. Vacuolation was not seen in any of the recovery dogs.

4. Hyaline droplets in hepatocytes (very slight to slight) was seen in 3/8 HD at terminal sacrifice; it was not seen in other groups. In the recovery dogs, however, it was seen in 2/4 controls, as well as in 3/4 LD, 0/3 MD, and 1/3 HD. It was not reported in dogs sacrificed at 6 months or in dogs which died during the study.

5. Bile duct proliferation was seen at the 6 month sacrifice in 0/4 controls, 0/4 LD, 2/4 MD (very slight to slight), and 2/4 HD (slight). At termination, it was seen in 5/8 HD (very slight to slight), not seen in other groups. After recovery period, seen in 1/4 controls (very slight), 2/4 LD (very slight), 0/3 MD, and 2/3 HD (very slight to slight). It was not seen in the 3 dogs which died during the study.

b. Kidney - At termination, brown pigment in tubular epithelium was seen in 3/8 controls, 6/8 LD, 5/8 MD, and 7/8 HD. The severity was greater (very slight to moderate) in the treatment groups, but not dose related. It was not seen at the 6 month sacrifice, or in dogs which died during the study. Among recovery dogs, it was seen in 3/4 controls, 2/4 LD, 3/3 MD, and 3/3 HD, the severity being very slight in controls and LD, slight to moderate in MD, and ranged from very slight to marked at HD.

c. Among the 3 dogs which died during the study, congestion and/or hemorrhage was found in several organs.

MUTAGENICITY

A. Ames Test

1. Plate Incorporation Assay

Salmonella strains TA 98, 100, 1535, 1537 and 1538 were used. Drug levels were 60-6000 μg per plate, with and without metabolic activation (Arocior - induced rat liver S9 preparation). Bupropion was weakly positive in the following strains:

a. TA 100, without metabolic activation: at all doses, dose-related; largest increase about 2x control. Positive control (1, 3 propane sultone, 0.04 μl per plate) caused 6.5x increase.

b. TA 100, with metabolic activation: at all doses, roughly dose related; largest increase less than 2x control. (Similar results in 2 separate studies). Positive control (2 aminoanthracene, 1 μg per plate) caused 9x increase.

c. TA 1535, with metabolic activation: in one study, dose-related increase starting at 300 μg, greatest effect 2x control; in a second study, non-dose-related increase at all doses, greatest effect 3x control. Positive control (2 aminoanthracene, 1 μg per plate) caused 10x increase.

2. Preincubation assay

Same Salmonella strain as above. Drug levels used were 15-1800 μg per plate (25-3000 μg/ml), with and without metabolic activation. Bupropion was weakly positive in the same strains in which it was positive in the plate incorporation assay, above:

a. TA 100, without activation: dose-related increase at 300-900 μg (1800 μg apparently bacteriotoxic); largest effect less than 1.5x control. Positive control (1,3 propane sultone, 0.04 μl per plate) caused 8x increase.

b. TA 100, with activation: generally dose related increase at 150 μg and above; largest effect less than 1.5x control. (Similar results in 2 separate studies.) Positive control (2 aminoanthracene, 1 μg per plate) caused 6x increase.

c. TA 1535, with activation: non dose-related increase at 150 μg and above; largest increase about 2x control. (Similar results in 2 separate studies.) Positive control (2 aminoanthracene, 1 μg per plate) caused 6x increase.

B. Rat Bone Marrow Chromosome Study

5M + 5F were given 0, 100, 200, or 300 mg/kg/day for 5 days by gavage. Positive control: 5M + 5F at 0.4 mg/kg i.p. triethylenemelamine.

No significant effects seen at LD and MD. At HD there was an increase in all types of chromosomal aberrations tabulated, with no sex differences noted. The % of aberrant cells was 4.6, 5.8, 5.3, and 9.3 in C, LD, MD, and HD, respectively. In contrast, the positive control produced 27.0% aberrant cells. The average number of aberrations per cell was also increased at HD: 0.050, 0.062, 0.067, and 0.140 in C, LD, MD, and HD, respectively. The value for positive control was 0.926. The mitotic index was decreased at HD (1.2 vs 2.4 in control) but this was stated to be not statistically significant.

C. DNA Binding

Rats were given either bupropion-C^{14} (100 mg/kg p.o.) or 2-AAF-C^{14} (20 mg/kg p.o.) and sacrificed 24 hr. later. The degree of covalent binding to liver DNA, RNA, and protein was then assessed. On a specific activity basis, 20x more 2-AAF equivalents were bound to DNA than that found for bupropion (despite the 5x higher dose of bupropion). A covalent binding index was calculated and compared with literature values for known hepatocarcinogens and non-hepatocarcinogens, and the value for bupropion was more similar to those in the latter category. The degree of binding to protein or RNA was not greatly different between bupropion and 2-AAF. It was concluded that the binding of bupropion to DNA, RNA, and protein was minimal and probably non-specific.

TWO GENERATION REPRODUCTION AND FERTILITY STUDY IN RATS

A. Method

1. Strain: Long-Evans

2. Dosage: 15 M and 30 F at 0 (2 groups - one vehicle treated, one untreated), 100, 200, or 300 mg/kg/day, by gavage, from day 60 pre-mating through mating (M) or from 15 days pre-mating through either day 13 of gestation (1/2 F) or day 21 postpartum (remaining F).

3. Procedure: Mating ratio was 2 F/M of the same dosage group. One of the 2 F mated to each M was sacrificed day 13 of gestation; the other F was allowed to deliver normally. Pup weights and survival were monitored to day 21 PP. At 12 weeks of age, these F1 generation pups were mated (1 F per litter mated to an intragroup M, but not a brother) and allowed to rear young (F 2 generation) to day 21 PP, during which time pup weights and survival were monitored.

B. Results

1. Observed signs

No drug effect in M; 1/30 MD F and 1/29 HD F had wobbly gait on days 1-2 of premating period only

2. Mortality

1 LD M, 1 MD M, and 1 HD F (dosing accidents), and 1 untreated control

3. Bodyweight

a. M - All drug treated groups gained more weight than vehicle controls, but not dose-related.

b. F - All drug teated groups gained slightly more weight than controls during mating period (not dose-related); this difference persisted through pregnancy and lactation periods.

4. Mating performance

a. M - No drug effect

b. F - No drug effects (pregnancy rate = 26/29, 24/30, 24/28, 22/30, and 23/29 in untreated controls, vehicle controls, LD, MD, and HD respectively).

5. Post-mortem uterine findings in F

   a. F sacrificed day 13 of pregnancy - no drug effects on number of total, live, or dead implants/dam, number of CL, or CL/implantation ratio.

   b. F which had not delivered by day 26 of gestation (4 vehicle controls, 3 LD, 3 MD, and 3 HD) - all had failed to implant (no implant scars seen).

   c. Pup - bearing F sacrificed day 21 PP - No drug effects on number of uterine scars.

6. Term deliveries

   No drug effects on number of live pups per dam. Slight increase in number of dead and live + dead pups per dam at HD.

7. Pup survival (F 1 generation)

   No drug effects through day 21 PP

8. Pup weight (F 1 generation)

   No drug effects through day 21 PP

9. Observed signs (F 1 generation)

   Results given up to 12 weeks of age - no drug effects

10 Bodyweight (F 1 generation)

   a. M - Slightly higher than vehicle controls at LD and HD (measured up to 12 weeks of age).

   b. F - No drug effect up to 12 weeks of age. During pregnancy, weight gain at HD was slightly decreased. No drug effect during lactation period.

11. Reproductive performance in M (F 1 generation)

   No drug effect on percent of M mating (100% in all groups) or siring litters (93, 64, 82, 82, and 90% in untreated controls, vehicle controls, LD, MD, and HD, respectively).

12. Gross necropsy in F 1 males at 12 weeks of age.

   No drug effects.

13. Reproductive performance in F (F 1 generation)

    a. No drug effect on pregnancy rate (7/11, 9/11, 9/12, and 9/11 in vehicle controls, LD, MD, and HD, respectively)

    b. No drug effect on length of gestation

    c. Slight decrease in mean number of live pups at HD compared to vehicle but not untreated controls; no drug effect on number of dead pups.

    d. Slight decrease in number of implant scars at HD.

14. Gross necropsy findings in F (F 1 generation).

    No drug effects among F not selected for mating (sacrificed at 12 weeks of age) or among pup-bearing F (sacrificed day 21 PP). No drug effect among F which were mated but had not delivered by day 26 of gestation - all were not pregnant and had no implant scars.

15. Pup survival (F 2 generation)

    No drug effects through day 21 PP.

16. Pup weight (F 2 generation)

    Mean weights slightly higher than controls at most days measured in all drug groups, not dose-related.

17. Number of pups with both eyes open on day 14 PP (F 2 generation)

    No drug effects.

18. Gross necropsy of F 2 pups (day 21 PP) - no drug effects.