CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

021164Orig1s000

NON-CLINICAL REVIEW(S)

PHARMACOLOGY/TOXICOLOGY MEMO TO THE FILE

NDA 21-164.
Submission N-000, AZ (Major amendment, multidisciplinary); stamp-dated 5/3/07.

Drug: gepirone HCl (Org 33062) Extended Release Tablets.
Sponsor: Fabre-Kramer Pharmaceuticals, Inc. [originally sponsored by Organon].
Indication: Major Depressive Disorder.

Reviewer: Linda H. Fossom, Ph.D., Pharmacologist.
HFD-130.

RE: Fabre-Kramer's Complete Response to the Agency's Action (2nd Not-Approvable) Letter dated 6/23/04. There are no Pharmacology/Toxicology issues that will affect approval (except for labeling negotiations).

Table of Contents for this Review:

1 BACKGROUND:

NDA 21-164 was originally submitted by Organon, Inc., on 9/30/99 (but was considered inadequate for filing) and subsequently resubmitted on 5/18/01, for the use of gepirone HCl as treatment for Major Depressive Disorder. The Pharmacology/Toxicology Reviewer for that submission (who is also the current Reviewer) recommended that the NDA was Approvable, contingent only upon a Phase IV commitment by the Sponsor to repeat the *in vitro* chromosomal aberration test, which was not adequate by current standards; and a description of the test as inadequate (though negative) (b) (4). [As excerpted from the original Pharmacology/Toxicology review (dated 3/8/02): "In the submitted study, gepirone was negative for 5-hr treatment, with and without metabolic activation. However, the study was not valid, because this negative finding (without activation) should have been followed up with a study using continuous treatment with gepirone (without activation) for ~24 hr (1.5 cell doubling times), in accordance with the current ICH Guidance." Recommended labeling for "Mutagenesis" was: "(b) (4)

(b) (4)

After secondary and tertiary reviews, it was decided that repeating the *in vitro* chromosomal aberration test would not be necessary, because gepirone was not mutagenic or clastogenic in the other 2 tests from the Standard Battery, as specified in the current ICH Guidance (1997) (and not mutagenic in 2 other *in vitro* tests) and was not carcinogenic in rat or mouse 2-year bioassays. However, it was decided that the results of the submitted *in vitro* chromosomal aberration test (b) (4) unless or until it was repeated in compliance with current standards. This was communicated to the Sponsor in the Not Approvable Letter (dated 3/15/02), not as a non-approval deficiency, but under "Other Requests and Comments" as excerpted below:

> **PRECLINICAL TOXICOLOGY**
>
> The *in vitro* chromosomal aberration assay was inadequate because although gepirone was negative for 5-hour treatment, with and without metabolic activation, this negative finding (without activation) should have been followed up with a study using continuous treatment with gepirone (without activation) for ~ 24 hours (1.5 cell doubling times) in accordance with current guidelines. Since the weight of evidence suggests that gepirone is neither genotoxic nor carcinogenic, we are not requiring that this study be repeated; however, it will have to be repeated if it is to be included in product labeling.

In their full response (AZ, stamp-dated 12/23/03) to that first Not Approvable letter, the Sponsor responded that "(b) (4) " This adequately answered the Pharmacology/Toxicology request as it was communicated in that Not Approvable Letter. However, based on clinical deficiencies, a second Not Approvable letter issued 6/23/04.

On 6/28/05, the Agency was notified that the ownership of this NDA had been transferred to Fabre-Kramer Pharmaceuticals, Inc.

The current submission (AZ, stamp-dated 5/3/07) provides Fabre-Kramer's complete response to the second Not Approvable letter, dated 6/23/04. Although there were no Pharmacology/Toxicology issues, the Sponsor has provided a report for a new in vitro chromosomal aberration test and included this study (b) (4).

2 THE CURRENT SUBMISSION:

The current submission is electronic and appears to contain all non-clinical studies that have been provided to this NDA: those provided in the original submission and in the 2003 submission (see Appendix B), as well as studies newly submitted here. In their current summary (designated summary-2007.pdf; under section E. Nonclinical Pharmacology and Toxicology Summary), the Sponsor notes only 2 new non-clinical studies conducted since the 2003 NDA Amendment: 1) study 030300 (final report issued 12/15/2003): an acute toxicity study of ORG 33062 (gepirone HCl) in male and female juvenile Sprague-Dawley rats; and 2) study 82/120: an in vitro study of Org 33062 for induction of chromosomal aberrations in cultured human peripheral blood lymphocytes. Only the in vitro chromosomal aberration assay will be considered here. [The Sponsor has already been given feedback on their proposed juvenile rat study, presumably based on this acute toxicity study (in an e-mail dated 4/7/03, under IND 33,626, N-123), however, it appears that the report for their juvenile toxicity study has not yet been submitted under that IND or this NDA.]

3 SUMMARY OF THE RESULTS OF THE NEW IN VITRO CLASTOGENICITY STUDY:

[Study 82/120: *An in vitro study of Org 33062 for induction of chromosomal aberrations in cultured human peripheral blood lymphocytes* is reviewed in detail in Appendix A; only a brief summary of that review is provided here.]

The Sponsor has provided a report for a human lymphocyte assay to assess the potential of gepirone HCl to induce chromosomal aberrations in vitro. For this study to be considered adequate, the following conditions would need to have been adequately tested: 1) a short treatment without metabolic activation; 2) a longer treatment without metabolic activation, if the short treatment was negative; and 3) a short treatment with metabolic activation. The study comprised the following treatments and results:

#1: **3-hr treatment without S9** (only tested in experiment 1): **negative** when tested up to a nominal concentration of 10 mM; this concentration would be considered adequate, even without cytoxicity**; however, the concentrations in the drug solutions should have been verified, particularly because of the lack of cytoxicity**.

#2: **20-hr treatment without S9** (only tested in experiment 2): **negative** up to 603.1 µg/ml, where cytotoxicity was evident (MI was decreased 57% compared with the negative control) **and adequate**.

#3: **3-hr treatment with S9** (tested in all 3 experiments): **equivocal/not clearly positive**; in experiment 1, it was not cytotoxic or clastogenic up to a nominal concentration of 10 mM (3595 µg/ml); in experiment 2, it was cytotoxic at 2876 (MI↓34%) and 3595 (MI↓68%) µg/ml and clastogenic only at the higher concentration; in experiment 3, it was cytotoxic at 2912 (MI↓19%) and 3236

(MI↓49%) and clastogenic at both concentrations [cytotoxicity was also evident at 3236 µg/ml (MI↓78%), but clastogenicity was not analyzed at that concentration].

Based on these findings, the 3-hr treatment without S9 could not be determined to be adequate: although the maximum (nominal) concentration of 10 mM would be considered to be adequate, the drug solutions apparently were not analyzed for drug content, so the actual concentrations were not verified; additionally there was no cytotoxicity that could indicate adequate dosing.

The 20-hr treatment without S9 is considered negative and adequate, based on observed cytoxicity; however, because the doses used were much lower than those that appeared to be tolerated when treatment was limited to 3 hrs, this 20-hr treatment cannot substitute for the inadequate 3-hr treatment. The 3-hr treatment with S9 is considered adequate but equivocal (i.e., not clearly positive), because of the variability among the 3 experiments where this condition was tested.

Additionally, it should be noted that the use of different sources of lymphocytes (from different subjects) for the different experiments in this study is not appropriate and would be expected to introduce uncontrolled and unnecessary variability into the results.

4 CONCLUSIONS/RECOMMENDATIONS:

There were no Pharmacology/Toxicology issues that would have prevented the approval of this NDA during the first or second review cycles. The only Pharmacology/Toxicology issue that was communicated to the original Sponsor (in the first Not Approvable letter dated 3/15/02), regarding the *in vitro* chromosomal aberration test that was not adequate by current standards, was adequately addressed by that Sponsor (in their response to that Not Approvable letter), who agreed (b) (4) (an option offered by the Agency). In the current submission, the response to our second Not Approvable letter (dated 6/23/04), the current Sponsor has provided a study report for a new in vitro chromosomal aberration test. This study has been reviewed here and found to be inadequate; (b) (4).

From a Pharmacology/Toxicology perspective, this NDA may be approved, (b) (4)

Additionally, the Sponsor should be informed that the use of different sources of lymphocytes (from different human subjects) for the different experiments in the currently submitted study is inappropriate and would be expected to introduce uncontrolled and unnecessary variability into the results.

As far as I am aware, we have not provided the Sponsor with labeling recommendations during the previous review cycles (both previous review cycles resulted in not-approvable decisions). However, my original review (dated 3/8/2002) provided recommendations for Pharmacology/Toxicology sections of labeling.

5 INFORMATION TO BE COMMUNICATED TO THE SPONSOR:

The human lymphocyte assay which you have submitted is not considered to have adequately tested gepirone HCl for in vitro clastogenicity; specifically it was not adequate for the short (3-hr) treatment time in the absence of metabolic activation. Although tested to a high nominal concentration of 10 mM, which would be considered adequate, the actual drug concentrations do not appear to have been verified by independent analysis and there was no clear cytotoxicity to indicate adequate dosing.

Because it was not considered necessary (in our first Not Approvable letter dated 3/15/02) that the original (but inadequate) in vitro clastogenicity study be repeated, we will not require that this inadequate study be repeated. However, (b) (4) .

Additionally, it should be noted that the use of lymphocytes from different subjects for the different experiments in this study is not considered appropriate, as it would be expected to introduce uncontrolled and unnecessary variability into the results.

6 SIGNATURES:

Linda H. Fossom, Ph.D., Pharmacologist *{see appended electronic signature page}*
Barry Rosloff, Ph.D., Supervisor *{see appended electronic signature page}*

7 APPENDIX A: REVIEW OF NEW IN VITRO CHROMOSOMAL ABERRATION ASSAY.

APPEARS THIS WAY ON ORIGINAL

Study title: Org 33062: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes.

[The CHO assay from the original submission did not show any evidence of chromosomal aberrations up to the HD of 1000 µg/ml, ±S9, for 5 hr; however, was considered inadequate, because a longer treatment without S9 was not conducted.]

Study no: (b) (4) study no.: 82/120; (b) (4) report no.: 82/120-D6172; Sponsor (N.V. Organon, The Netherlands) no.: 0300202; report issued January 2004.
Study type: *In vitro* chromosomal aberration test.
Volume #, and page #: electronic file [030202.pdf; 55 pages.].
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/21/03, with experimental work conducted on 5/29-9/5/03.
GLP compliance: yes, see page 2 of study report.
QA reports: yes, see page 3 of study report.
Drug, lot #, and % purity: Org 33062 (gepirone HCl), batch number 0102001, 100.1% pure (by HPLC; Organon's C/A report dated 12/13/02).

Formulation/vehicle: water for injection, with membrane filter-sterilization (0.2 um).

Methods:

Cell line: primary peripheral lymphocytes from 3 healthy, non-smoking female volunteers were pooled for each of 3 experiments (from a total of 7 subjects).
Test drug: Org 33062 (gepirone HCl) was dissolved in purified water (using vortex mixing in experiment 1 only) to 39.95 mg/ml (10 mM), filter-sterilized (0.2 um), and further diluted using sterile purified water.
Dose selection criteria:

Basis of dose selection: According to the study report, geprione HCl was soluble in water for injection to at least 52.74 mg/ml, which would result in a final concentration of 5274 µg/ml after 10-fold dilution into culture medium; there was no visible precipitation at that concentration. However, a top concentration of 3595 µg base/ml (10 mM) was chosen. [A high concentration of 10 mM is appropriate for mammalian cell assays, according to ICH S2A Guideline for Industry: Specific aspects of regulatory genotoxicity tests for pharmaceuticals (April 1996).)]

Range finding studies: not conducted (see basis for dose selection, above).

Test agent stability: according to the report, "The test article solutions were protected from light and used within 2.5 hours of initial formulation…" Apparently, solutions were not analyzed; "Determinations of stability and characteristics of the test article were the responsibility of the Sponsor."
Metabolic activation system: S9 (post-mitochondrial fraction) from livers of male Sprague-Dawley rats, induced with Aroclor 1254 ((b) (4) ; quality control certificate in Appendix 7, indicated

mutagenic activity using 5 µg benzo(a)pyrene or 2.5 µg 2-AA); used at 2% S9 in final incubation.

Controls:

Vehicle: sterile water for injection.

Negative controls: vehicle.

Positive controls: without metabolic activation: 4-nitroquinoline 1-oxide (NQO at 1.25, 2.50, 5.00 µg/ml, but only data for 2.5 (experiments 1 and 2) analyzed/presented); with activation: cyclophosphamide (CPA at 3.125, 6.25, 12.5 µg/ml, but only data for 6.25 (experiment 1) and 3.125 (experiments 2 and 3) analyzed/presented). Positive controls were dissolved in DMSO and diluted 100-fold into culture medium.

Exposure conditions:

Incubation and sampling times: cells were incubated with drug with or without S9 for 3h or without S9 for 17 hr, then washed, and further incubated with fresh medium for a total treatment time of ~20 hr; cultures were treated with colcemid for ~2 hours before harvest (i.e, before metaphase spreads were prepared).

Three independent experiments were conducted (see table, below).

Table 1. Treatment conditions for 3 experiments testing 3-hr treatment ±S9 (experiment 1), 20-hr –S9 and 3-hr +S9 (experiment 2), and 3-hr +S9 (experiment 3). [Excerpted directly from page 18 of the study report.]

Treatment	S-9	Number of cultures 3+17*	20+0*
Experiment 1			
Negative control	-	4	
	+	4	
Test article	-	2	
(all doses)	+	2	
Positive controls	-	2	
(all doses)	+	2	
Experiment 2			
Negative control	-		4
	+	4	
Test article	-		2
(doses as appropriate)	+	2	
Positive controls	-		2
(all doses)	+	2	
Experiment 3			
Negative control	-		
	+	4	
Test article	-		
(all doses)	+	2	
Positive controls	-		
(all doses)	+	2	

* Hours treatment + hours recovery

Doses used in definitive study: 3 experiments were conducted and the doses used in each are presented in table 2, below.

Analysis:

No. of replicates: (for each of the 3 experiments) duplicate flasks for positive controls and for each concentration of test drug; quadruplicate flasks for negative controls (however, only 2 negative control flasks were analyzed for aberrations, but all 4 for mitotic index).

Counting method: 1000 cells/flask were assessed for mitotic index, vs negative control; 100 metaphases were analyzed per treatment flask (i.e., per replicate); aberrant cells in each culture were categorized as follows: 1) cells with structural aberrations including gaps, 2) cells with structural aberrations excluding gaps, and 3) polyploidy, endoreduplicated or hyperdiploid cells.

Criteria for positive results: quoting from the Sponsor's protocol (page 22 of 55):
"

A test article is considered as positive in this assay if:

1. the proportions of cells with structural aberrations at one or more concentration exceeds the normal range in both replicate cultures, and

2. a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurs at these doses.

"

Summary of individual study findings:

- **3-hr treatment without S9** (experiment 1)**: negative;** no evidence of increased number of cells with aberrations (including or excluding gaps) when analyzed at nominal concentrations of 2022, 2696, and 3595 µg/ml (i.e., **10 mM**), with **no significant cytotoxicity** (mean MIs ≥80% of negative control); **however, drug solutions were not analyzed for drug content.**
- **20-hr treatment without S9** (experiment 2): **negative;** no evidence of increased number of cells with aberrations when analyzed at nominal concentrations of 386, 482.5 and 603.1 µg/ml, with evidence of significant cytotoxicity (mean MIs were decreased 23%, 43%, and 57%, respectively, compared with negative control).
- **3-hr treatment with S9** (experiment 1, 2, and 3; see table 2, below): **not clearly positive**; negative up to 3595 µg/ml (i.e, 10 mM), with mean MI ≥86% of negative control (experiment 1); **but** cells with aberrations (with or without gaps) were increased at 3595 µg/ml, where mean MI was 68% lower than negative control, but not at ≤2876 µg/ml, where mean MI was ≤33% lower than negative control (experiment 2); **and** cells with aberrations (with or without gaps) were increased at 2912 and 3595 µg/ml, where mean MI was 19 and 48% lower than negative control, respectively, but not at 2123 µg/ml, where mean MI was not different from negative control (experiment 3).

Table 2. The dose-response to gepirone HCl in the presence of S9 (for 3 hr) on cytotoxicity (decreased mitotic index, compared with vehicle control) and clastogenicity (increased number of cells with aberrations, excluding gaps, based on 100 cells per duplicate culture). Square brackets, [], indicate that aberrations were not analyzed. [Compiled from information on MI presented in tables from pages 23, 24, and 25 of the study report; and clastogenicity identified in tables 1- 5, pages 33-37.]

[DRUG], µG/ML	EXP 1		EXP 2		EXP 3	
	↓MI	Ab cells	↓MI	Ab cells	↓MI	Ab cells
solvent		2/1		0/0		3/1
853.1	[1]					
1137	[4]					
1178			[5]			
1473			12	0/1		
1517	[16]					
1841			[24]			
2022	14	1/1				
2123					0	1/3
2301			[30]			
2359					[3]	
2621					[3]	
2696	10	2/1				
2876			34	3/0		
2912					19	5/13
3236					49	11/14
3595	9	2/4	68	18/16	[78]	
Positive-control		26/33		14/31		42/45

In the 2 experiments where gepirone HCl induced aberrations after 3 hr treatment in the presence of S9, with largest increases in the number of chromatid deletions, with smaller increases in chromosome deletions, gaps, and chromatid exchanges, similar to the findings for the positive control CPA (see table 3, below).

Table 3. Gepirone HCl in the presence of S9 (for 3 hr) increased chromosome deletions, chromatid deletions, chromatid exchanges, and gaps, similar to the positive control CPA. [Excerpted from table 9 (experiment 2) and table 10 (experiment 3] pages 42 and 43 of the study report.]

Table 9
3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2
Donor sex: female

Treatment (µg/mL)	Rep	Cells *	G	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Solvent	A	100	2	0	0	0	0	0	2	0
	B	100	0	0	0	0	0	0	0	0
	Total	200	2	0	0	0	0	0	2	0
1473	A	100	1	0	0	0	0	0	1	0
	B	100	0	0	0	1	0	0	1	1
	Total	200	1	0	0	1	0	0	2	1
2876	A	100	1	0	0	4	0	0	5	4
	B	100	0	0	0	0	0	0	0	0
	Total	200	1	0	0	4	0	0	5	4
3595	A	100	6	5	0	20	7	1	39	33
	B	100	9	4	0	17	3	2	35	26
	Total	200	15	9	0	37	10	3	74	59
CPA, 3.125	A	66	6	11	0	9	1	0	27	21
	B	80	10	14	0	38	5	0	67	57
	Total	146	16	25	0	47	6	0	94	78

* Total cells examined for structural aberrations
Totals given for each culture may differ from values given in Appendix 1, Tables 1 - 5 if cells are observed which have more than one aberration
For abbreviations and classification see Appendix 2

Table 10
3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 3
Donor sex: female

Treatment (µg/mL)	Rep	Cells *	G	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Solvent	A	100	0	0	0	3	0	0	3	3
	B	100	0	0	0	2	0	0	2	2
	Total	200	0	0	0	5	0	0	5	5
2123	A	100	0	0	0	1	0	0	1	1
	B	100	0	0	0	3	0	0	3	3
	Total	200	0	0	0	4	0	0	4	4
2912	A	100	1	1	0	7	4	0	13	12
	B	100	8	1	0	15	1	0	25	17
	Total	200	9	2	0	22	5	0	38	29
3236	A	100	6	4	0	11	2	0	23	17
	B	100	12	1	0	24	1	0	38	26
	Total	200	18	5	0	35	3	0	61	43
CPA, 3.125	A	100	13	14	0	49	6	2	84	71
	B	100	19	15	0	57	6	0	97	78
	Total	200	32	29	0	106	12	2	181	149

* Total cells examined for structural aberrations
Totals given for each culture may differ from values given in Appendix 1, Tables 1 - 5 if cells are observed which have more than one aberration
For abbreviations and classification see Appendix 2

[It should be noted that an increase in the percentage of cells with numerical aberrations was seen (in experiment 3) at 2912 (3.4%) and 3236 (6.5%) µg/ml +S9; this was due to polyploidy. Significant incidences of numerical aberrations were not seen with gepirone HCl at other concentrations or conditions or experiments or in positive controls.]

Study validity: **not valid for 3-hr treatment without S9**: The experiments in this study appeared valid for 3-hr treatment with S9 and 20 hr treatment without S9 (adequate positive controls, adequate durations of dosing, evidence of cytotoxicity) and on face for 3-hr treatment without S9 (because it was tested up to a nominal concentration of 10 mM). However, the drug content of the drug solutions was not analyzed and this was critically important for the 3-hr treatment without S9, because there was no evidence of cytotoxicity.

Additionally, there was indirect evidence that the experiment where 3-hr treatment without S9 was tested (experiment 1) might have been sub-optimal. Careful consideration of the concentration-related cytoxicity in the presence of S9 in all 3 experiments (see table, below) shows a dramatic difference in the dose-responses, with no significant dose-

response in experiment 1 up to the maximum nominal concentration of 3595 µg/ml (i.e., 10 mM); a very steep dose-response in experiment 3, rising from no toxicity at 2621 µg/ml to 78% toxicity at 3595 µg/ml; and a dose-response of intermediate slope in experiment 2, going from no toxicity at 1178 µg/ml to 30% toxicity at 2301 µg/ml, and 68% toxicity at 3595 µg/ml. The lack of cytotoxicity after 3-hr treatment with S9 at concentrations up to 3595 µg/ml in experiment 1, when concentrations as low as 1841 µg/ml (experiment 2) and 2912 µg/ml (experiment 3) were cytotoxic in other experiments, is consistent with lack of sensitivity in experiment 1, a finding that could be true for the 3-hr treatment without S9 as well. [There were 2 obvious confounding variables across the 3 experiments: 1) the drug solutions (which were not analyzed for drug content); and 2) the source of the lymphocytes (lymphocytes used for each experiment were pooled from 3 subjects, but across the 3 different experiments, the pools were obtained from 3 different subjects or 2 different and 1 common subject).]

Table 4. The dose-response for cytotoxicity (decreased mitotic index, compared with vehicle control) to gepirone HCl in the presence of S9 varied across experiments. Asterisks indicate increased clastogenicity (increased cells with aberrations, excluding gaps). Square brackets, [], indicate that aberrations were not analyzed. [Compiled from information on MI presented in tables from pages 23, 24, and 25 of the study report; and clastogenicity identified in tables 1- 5, pages 33-37.]

CONC, µg/ml	↓ MI, vs vehicle (%)		
	exp 1	exp 2	exp 3
(donors)	((b) (6))	((b) (6))	((b) (6))
853.1	[1]		
1137	[4]		
1178		[5]	
1473		12	
1517	[16]		
1841		[24]	
2022	14		
2123			0
2301		[30]	
2359			[3]
2621			[3]
2696	10		
2876		34	
2912			19*
3236			49*
3595	9	68*	[78]

Conclusions/Recommendations:

The Sponsor's conclusions: The Sponsor concluded that: 1) 3-hr and 20-hr treatments in the absence of S9 resulted in clear negative responses, even at concentrations that resulted in more than 50% cytotoxicity; 2) in the presence of S9, positive responses were only seen at high concentrations, near the upper limit of 10 uM and at which cytotoxicity

was evident (and in only 2 out of 3 experiments). Although the difference in the toxicity profile for +S9 in experiment 1 vs 2 and 3 was noted in the results section, with the comment that "The reason for this shift in toxicity was not clear, but variability between experiment is often observed where there are steep toxicity responses close to the upper limit of testing."

(b) (4) refers to this study (the sited reference 164 is for (b) (4) study report no. 82/120, which is the study currently under review) as follows: "(b) (4)

This Reviewer's conclusions: For this study to be considered adequate, three conditions would need to have been adequately tested: 1) 3-hr treatment without S9, 2) 20-hr treatment without S9, and 3) 3-hr treatment with S9.

#1: **3-hr treatment without S9** (only tested in experiment 1): **negative** when tested up to a nominal concentration of 10 mM; this concentration would be considered adequate, even without cytoxicity**; however, the concentrations in the drug solutions should have been verified, particularly because of the lack of cytoxicity**.

#2: **20-hr treatment without S9** (only tested in experiment 2): **negative** up to 603.1 µg/ml, where cytotoxicity was evident (MI was decreased 57% compared with the negative control) **and adequate**.

#3: **3-hr treatment with S9** (tested in all 3 experiments): **not clearly positive**; in experiment 1, it was not cytotoxic or clastogenic up to 10 mM (3595 µg/ml); in experiment 2, it was cytotoxic at 2876 (MI↓34%) and 3595 (MI↓68%) µg/ml and clastogenic only at the higher concentration; in experiment 3, it was cytotoxic at 2912 (MI↓19%) and 3236 (MI↓49%) and clastogenic at both concentrations [cytotoxicity was evident at 3236 µg/ml (MI↓78%), but clastogenicity was not analyzed at that concentration].

Based on these findings, the 3-hr treatment without S9 could not be determined to be adequate; although the maximum nominal concentration of 10 mM would be considered to be adequate, the drug solutions apparently were not analyzed, so the actual concentration was not verified; additionally there was no cytotoxicity that could indicate adequate dosing.

The 20-hr treatment without S9 is considered negative and adequate; however, because the doses used were much lower than those that appeared to be tolerated when treatment was limited to 3 hrs, this 20-hr treatment cannot substitute for the inadequate 3-hr treatment. The 3-hr treatment with S9 is considered adequate but equivocal (i.e., not clearly positive), because of the variability among the 3 experiments.

Additionally, the Sponsor should be informed that the use of different sources of lymphocytes (from different subjects) for the different experiments would be expected to introduce uncontrolled and unnecessary variability into the results.

Information to be communicated to the Sponsor:

The human lymphocyte assay which you have submitted is not considered to have adequately tested gepirone HCl for in vitro clastogenicity; specifically it was not adequate for the short (3-hr) treatment time in the absence of metabolic activation. Although tested to a nominal high concentration of 10 mM, which would be considered adequate, the actual drug concentrations do not appear to have been verified by independent analysis and there was no clear cytotoxicity to indicate adequate dosing.

[Because it was not considered necessary (in our first Not Approvable letter dated 3/15/02) that the original (but inadequate) in vitro clastogenicity study be repeated, we will not require that this inadequate study be repeated. (b) (4)

Additionally, it should be noted that the use of lymphocytes from different subjects for the different experiments in this study is not considered appropriate, as it would be expected to introduce uncontrolled and unnecessary variability into the results.

8 APPENDIX B: TABLE OF CONTENTS FOR 2003 SUBMISSION:

Pharmtox Table of Contents NPT Summary of Org 33062		
Description	Review Copy Volume Number	Archive copy Folder\File Name
2003 Submission		
II. Pharmacology studies		
a. Pharmacodynamics		
AN4516 (NL0047818)	n/a	Pharmtox\pharm\AN4516-2003.pdf
AN4521 (NL0036376)	n/a	Pharmtox\pharm\AN4521-2003.pdf
III. Pharmacokinetics		
AN2791 (NL0037524)	n/a	Pharmtox\pk\AN2791-2003.pdf
AN2899 (NL0032235)	n/a	Pharmtox\pk\AN2899-2003.pdf
V. Publications		
Literature Search	n/a	Pharmtox\pubs\litsearch-2003.pdf

N/A=Not available

AN4516 (NL0047818): Org 33062 and Org 25907 – Evaluation of effect on cardiac action potential in isolated canine Purkinje fibres.

AN4521 (NL0036376): ORG 33062: Evaluation of effects on blood pressure, heart rate and electrocardiogram after single oral administration to conscious dogs.

AN2791 (NL0037524): In vitro binding or Org 25907 to male mouse, rat, rabbit, dog and human plasma proteins.

AN2899 (NL0032235): An oral single and multiple dose toxicokinetic study with Org 33062 in Beagle dogs.

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/s/

Linda Fossom
10/10/2007 01:36:15 PM
PHARMACOLOGIST

Barry Rosloff
10/12/2007 05:26:43 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY MEMO TO THE FILE

NDA 21-164.
Submission N-000, AZ (Major amendment, multidisciplinary); stamp-dated 12/23/03.

Drug: gepirone HCl (Org 33062) Extended Release Tablets.
Sponsor: Organon, Inc.
Indication: Major Depressive Disorder.

Reviewer: Linda H. Fossom, Ph.D., Pharmacologist.
HFD-120.

RE: Organon's Complete Response to the Agency's Action (Not-Approvable) Letter dated 3/15/03.

Background: NDA 21-164 was originally submitted on 9/30/99, for the use of gepirone HCl as treatment for Major Depressive Disorder. The Pharmacology/Toxicology Reviewer for that submission (who is also the current Reviewer) recommended that the NDA was Approvable, contingent only upon a Phase IV commitment by the Sponsor to repeat the *in vitro* chromosomal aberration test, which was not adequate by current standards; and a description of the test as inadequate (though negative) (b) (4). [As excerpted from the original Pharmacology/Toxicology review (dated 3/8/02): "In the submitted study, gepirone was negative for 5-hr treatment, with and without metabolic activation. However, the study was not valid, because this negative finding (without activation) should have been followed up with a study using continuous treatment with gepirone (without activation) for ~24 hr (1.5 cell doubling times), in accordance with the current ICH Guidance." Recommended labeling for "Mutagenesis" was: "(b) (4)]

After secondary and tertiary reviews, it was decided that repeating the *in vitro* chromosomal aberration test would not be necessary, because gepirone was not mutagenic or clastogenic in the other 2 tests from the Standard Battery, as specified in the current ICH Guidance (1997) (and not mutagenic in 2 other *in vitro* tests) and was not carcinogenic in rat or mouse 2-year bioassays. However, it was decided that the results of the submitted *in vitro* chromosomal aberration test (b) (4) unless or until it was repeated in compliance with current standards. This was

communicated to the Sponsor in the Not Approvable Letter (dated 3/15/02), not as a non-approval deficiency, but under "Other Requests and Comments" as excerpted below:

> **PRECLINICAL TOXICOLOGY**
>
> The *in vitro* chromosomal aberration assay was inadequate because although gepirone was negative for 5-hour treatment, with and without metabolic activation, this negative finding (without activation) should have been followed up with a study using continuous treatment with gepirone (without activation) for ~ 24 hours (1.5 cell doubling times) in accordance with current guidelines. Since the weight of evidence suggests that gepirone is neither genotoxic nor carcinogenic, we are not requiring that this study be repeated; however, it will have to be repeated if (b) (4).

In this submission, the Sponsor responded that "(b) (4) " This adequately answers the Pharmacology/Toxicology request as it was communicated in the Not Approvable Letter (see excerpt, above).

Conclusions/recommendations:

There were no Pharmacology/Toxicology issues that would have prevented the approval of this NDA during the first review cycle. The only Pharmacology/Toxicology issue that was communicated to the Sponsor, regarding the *in vitro* chromosomal aberration test that was not adequate by current standards, was adequately addressed by the Sponsor, (b) (4) (an option offered by the Agency in the Not Approvable Letter dated 3/15/02).

From a Pharmacology/Toxicology perspective, this NDA can be approved (b) (4)).

Linda H. Fossom, Ph.D., Pharmacologist *{see appended electronic signature page}*
Lois Freed, Ph.D., Supervisor *{see appended electronic signature page}*

Cc:
PDavid

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/s/

Linda Fossom
4/7/04 03:30:42 PM
PHARMACOLOGIST

Lois Freed
4/7/04 03:52:42 PM
PHARMACOLOGIST

MEMORANDUM

To: File, NDA 21-164

Through: Robert Temple, M.D., ODE I Office Director
Russell Katz, M.D., Division Director, Neuropharmacologic Drug Products
Barry Rosloff, Ph.D., Pharmacology Supervisor, HFD-120
Linda Fossom, Ph.D, Pharmacology Reviewer, HFD-120
Paul David, R.Ph., Project Manager, HFD-120

From: Jeri El-Hage, Ph.D., ODE I Associate Director for Pharmacology/Toxicology

Subject: NDA 21-164 , Ariza, Gepirone HCl Extended Release Tablets
Tertiary Review of Pharmacology/Toxicology Data

Date: March 13, 2002

A complete toxicological evaluation of gepirone HCl has been conducted and the toxicity profile supports the recommendations of the pharmacology reviewer and team leader for NDA approval. However, I disagree with the reviewer's recommendation that an additional genotoxicity assay, namely a chromosome aberrations assay in CHO cells with 24 hour treatment, needs to be conducted as a Phase IV commitment. Gepirone HCl tested negative in 4 genotoxicity assays (Ames, *in vivo* rat micronucleus, hepatocyte DNA repair, and CHO/HGPRT mammalian gene mutation assays) and in a chromosome aberrations assay conducted in CHO cells with 5 hours treatment. More importantly, there was no evidence of carcinogenic potential in the completed two-year mouse and rat bioassays.
Based on a weight of evidence approach, the outcome of the recommended additional genotoxicity assay would not influence the overall conclusion regarding the genotoxic and/or carcinogenic potential of gepirone. Therefore, since the outcome of the recommended genotoxicity assay would have no influence on our regulatory decision, it does not seem warranted.

I do not know if the Division intends to communicate any labeling comments at this time.
The preclinical sections (i.e., fertility, mutagenicity, carcinogenicity, pregnancy category) of the sponsor's proposed labeling require major revision. The revisions proposed by the pharmacology reviewer , Dr Fossom, are generally acceptable. I agree that the findings in the reproductive toxicity studies support labeling as a Pregnancy Category C, (b) (4) . I defer to the Division to compare the results of the reprotoxicity studies for gepirone to other SSRIs or 5-HT1A agonists and determine the consistency of labeling for pregnancy category across the class. Regarding the mutagenicity section, I agree with Dr Rosloff's recommendation that (b) (4)

It is also noted that although the current practice is to express exposure multiples in the labeling based on AUC ratios, the pharmacokinetics and drug metabolism data provide a strong rationale for expressing exposure multiples in the toxicity studies on the basis of body surface area (Mg/M^2) as written.

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/s/

Jeri El Hage
3/13/02 04:05:05 PM
PHARMACOLOGIST

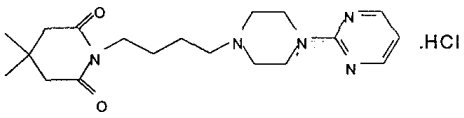
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: **NDA 21-164.**
Review number: 1.
Sequence number/date/type of submission: 000 / 5-18-01 / Original New Drug Application/Resubmission.
Information to sponsor: Yes (X) No ( ).
Sponsor and/or agent: Organon Inc., 375 Mount Pleasant Ave., West Orange, NJ 07052.
Manufacturer for drug substance: (b) (4)

Reviewer name: Linda H. Fossom.
Division name: Neuropharmacological Drug Prooducts.
HFD #: 120
Review completion date: 3/8/02.

Drug:

Code Name: Org 33062; MJ 13805-1; BMY-13805-1
Generic Name: **gepirone hydrochloride**
Trade Name: Ariza.
Chemical Name: 2,6-Piperidinedione, 4,4-dimethyl-1-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-, monohydrochloride.
CAS Registry Number: 83928-66-9.
Molecular Formula/ Molecular Weight: $C_{19}H_{29}N_5O_2 \cdot HCl$ / 395.9 g/mole.
Structure:



Relevant INDs/NDAs/DMFs: IND 23,952 and IND 33,626 (immediate- and extended-release tablets, respectively; Fabre-Kramer Pharmaceuticals, Inc); DMF (b) (4) (drug substance); several other INDs and DMFs.

Drug class: 5-HT_{1A} serotonin receptor agonist.

Indication: Treatment of Depression, starting with 20 mg/day dose, increasing to a maximum daily dose of 80 mg/day as needed.

Clinical formulation: extended-release tablets; proposed 20, 40, and 80 mg tablets.

Route of administration: oral.

Disclaimer: Tabular and graphical information is used directly from Sponsor's submission where feasible and is identified as such.

Executive Summary

I. RECOMMENDATIONS

A. Recommendation on Approvability:

Approvable, with Phase IV commitment for *in vitro* chromosomal aberration test, as described below.

B. Recommendation for Nonclinical Studies:

The *in vitro* chromosomal aberration test, part of the standard test battery according to the current ICH Guidance for Industry, S2B Genotoxicity, 1997, was inadequate and should be repeated. In the submitted study, gepirone was negative for 5-hr treatment, with and without metabolic activation. However, the study was not valid, because this negative finding (without activation) should have been followed up with a study using continuous treatment with gepirone (without activation) for ~24 hr (1.5 cell doubling times), in accordance with the current ICH Guidance.

Because gepirone was not mutagenic or clastogenic in the other 2 tests from the Standard Battery, as specified in the current ICH Guidance (1997) (and not mutagenic in 2 other *in vitro* tests) and was not carcinogenic in rat or mouse 2-year bioassays, a Phase IV commitment for this study would be acceptable.

C. Recommendations on Labeling: explanation, where necessary, noted in [].

1 Page(s) of Draft Labeling has been Withheld in Full as b4 (CCI/TS) immediately following this page

II. SUMMARY OF NONCLINICAL FINDINGS

A. Brief Overview of Nonclinical Findings: See "Summary and Conclusions" in section IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS, at the end of this review, below.

B. Nonclinical Safety Issues Relevant to Clinical Use: See recommended labeling above.

III. ADMINISTRATIVE

A. Reviewer signature: Linda H. Fossom
{see appended electronic signature page}

B. Supervisor signature: Concurrence - Barry Rosloff
{see appended electronic signature page, with signature comments}

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: *In vitro* and animal studies have shown gepirone to be a moderate affinity, selective agonist at 5-HT_{1A} subtype serotonin receptors. 5-HT_{1A} receptors are currently thought to act as presynaptic autoreceptors (linked through Gi-proteins to adenylate cyclase) and as postsynaptic receptors to regulate a potassium current(s) (through Go-proteins). The Sponsor claims that gepirone is an agonist at presynaptic receptors and a partial agonist at postsynaptic receptors, however, the evidence for partial vs full agonism is not compelling.

Mechanism of action: The mechanism of action of geprione as an anitdepressant is unknown. As for other currently approved antidepressants, the actual mechanism of action is probably through (as yet unidentified/unexplained) compensatory mechanisms initiated by the direct effect(s) of the drugs. For gepirone, the initiating effect is assumed to be its direct effects at 5-HT_{1A} receptors.

Secondary pharmacodynamics: Although geprione appears specific for 5-HT_{1A} receptors, with some potential activity ad D2 dopamine receptors, 1-PP (a major metabolite in humans) has antagonist activity at alpha-2 adrenergic (auto) receptors, increasing norepinephrine release and activity. The other major human metabolite, 3'-OH-gepirone, appears to be specific for 5-HT_{1A} receptors (with affinity similar to gepirone), in the limited number of receptor types tested. It would be of interest to know whether gepirone or metabolites has antagonist activity at MAO or biogenic amine transporters, such at serotonin reuptake transporter, like other classes of antidepressants. I could not find compelling evidence in the current submission regarding these issues: the evidence that gepirone did not inhibit these proteins was weak and there was some evidence that a major human metabolite (1-PP) blocked the serotonin transporter.

It should also be noted that compensatory changes at other points in the serotonergic system (e.g., down-regulation of 5-HT_2 receptors noted in rats) or in other systems may underlie pharmacological and toxicological responses after repeated administration of gepirone.

In vitro binding studies: From the Sponsor's summary table of *in vitro* binding data below, it appears that gepirone binds selectivity to the 5-HT_{1A} subtype serotonin receptor, with moderate affinity, and little if any affinity for the other receptors that are listed. Notably absent are nicotinic cholinergic receptors. The Sponsor seems to feel that gepirone has no meaningful affinity for dopamine receptors and contrasts this favorably with buspirone's apparently higher affinity. From the binding data referenced here, this is not clear. Gepirone does seem to have low or no affinity at D1 receptors (displacement of SCH 23390) and D2 receptors (displacement of spiperone) from rat brain or striatal membranes. However, in the studies using cloned receptors of the D2 class (i., D2, D3, and D4), there is some discrepancy. Gepirone had moderate affinity at cloned rat D2 receptors (Chio, 1990) with a Ki of 58 nM (vs U-86170). In human clones of D2-

type receptors, gepirone showed weak if any affinity at D2L and D2S forms, but moderate affinity at 2 other D2-type receptors (D3 and D4).

Table 1. Sponsor's summary table (from Supplement to the Nonclinical Pharmacology & Toxicology Summary) showing *in vitro* receptor binding affinities for gepirone.

Receptor Site	Ligand	IC_{50} or K_i (nM)	References
5-HT_1	5-HT	3770	(b) (4)-09729
5-HT_{1A}	8-OH-DPAT	54	-11156
		13	Piercy et al, 1994
		115	Yevich et al, 1990
		70	Hamik et al, 1990
		256	Hamon et. al., 1988
5-HT_{1A} (human clone)		38	(b) (4)NL 0025880
	5-MeO-DPAC	97	Cossery et a., 1987
5-HT_{1B}	5-HT	7800	(b) (4)-11156
		>100000	Hamik et al, 1990
5-HT_{1C}	Mesulergine	5000	Hamik et al, 1990
5-HT_{1D}	5-HT	>100000	Hamik et al, 1990
5-HT_2	Spiperone	21800	(b) (4)-09570 Yevich et al, 1990
		9000	(b) (4)-09729
		8000	-11164
		5875	McMillen et. al., 1987
	Ketanserin	3000	Hamik et al, 1990
5-HT_{2A} (human clone)	Ketanserin	>6310	(b) (4)NL 0025880
5-HT_{2C} (human clone)	Mesulergine	>3333	NL 0025880
5-HT_6 (human clone)	LSD	>50119	NL 0025880
5-HT_7 (human clone)	LSD	635	NL 0025880
D_1 Dopamine	SCH 23390	>1000	(b) (4)-25001 Yevich et al, 1990
		>100000	Hamik et al, 1990
D_2 Dopamine	Spiperone	1905	(b) (4)-09570 Yevich et al, 1990
		5400	(b) (4)-09729
		2200	Hamik et al, 1990

Receptor Site	Ligand	IC_{50} or K_i (nM)	References
		4200	(b) (4)-11164
		2985	McMillen et. al., 1987
	N-propylnorapomorphine	1150	(b) (4)-09570 Yevich et al, 1990
	U-86170	58	Piercy et al, 1994
D_{2L} (human clone)	Spiperone	876	(b) (4) NL 0025880
D_{2s} (human clone)	Spiperone	2630	NL 0025880
D_3 (human clone)	Spiperone	115	NL 0025880
D_4 (human clone)	Spiperone	193	NL 0025880
α_2-Adrenergic	Clonidine	10000	(b) (4)-09729
		8800	-11164
		>1042	Piercy et al, 1994
	Rauwolscine	1600	Hamik et al, 1990
α_{2A} (human clone)	Rauwolscine	3802	(b) (4) NL 0025880
α_{2B} (human clone)	Rauwolscine	12823	NL 0025880
α_{2C} (human clone)	Rauwolscine	1042	NL 0025880
α_1-Adrenergic	WB-4101	1960	(b) (4)-09570 Yevich et al, 1990
		480	(b) (4)-09729
		800	-11164
		2300	Hamik et al, 1990
	Prazocin	>2427	Piercy et al, 1994
β-Adrenergic	Dihydroalprenodol	>100000	(b) (4)09729 Yevich et al, 1990
		>100000	(b) (4)-11164
		>100000	Hamik et al, 1990
Muscarinic	Quinnuclidinyl benzilate (QNB)	21300	(b) (4)-09570 Yevich et al, 1990
		>100000	Hamik et al, 1990
		>100000	(b) (4)-25291
Muscarinic M_1	Pirenzepine	28800	-25291
Muscarinic M_2	Oxotremorine-M	>10000	-25291
Muscarinic M_1 (human clone)	Scopolamine	>45709	(b) (4) NL 0025880
Muscarinic M_2 (human clone)	Scopolamine	>53703	NL 0025880
Muscarinic M_3 (human clone)	Scopolamine	>42658	NL 0025880
Muscarinic M_4 (human clone)	Scopolamine	>51286	NL 0025880
Benzodiazepine	Diazepam	>1000	(b) (4)09570
Benzodiazepine	Flunitrazepam	>100000	-09653
		>100000	Hamik et al, 1990
GABA	GABA	>1000	(b) (4)-09657
$GABA_A$	Muscimol	>1000	(b) (4)-09250 Yevich et al, 1990
κ-Opiate	U-69,593	>1000	(b) (4)-25244
Opioid	Naloxone	>1000	Yevich et al, 1990
Cholecystokinin	Propionyl-CCK_8	>100000	(b) (4)-25091 Yevich et al, 1990
Imipramine	Imipramine	>1000	(b) (4)-09570 Yevich et al, 1990
H_1 Histamine	Pyrilamine	> 1000	Yevich et al, 1990
Glycine	Strychnine	>100000	(b) (4)-09570 Yevich et al, 1990
Kainic	Kainic Acid	10300	Yevich et al, 1990
NMDA	CPP	>100000	(b) (4)-25239
Quisqualate	AMPA	>100000	-25240
PCP	(+)MK-801	>100000	-25214 Yevich et al, 1990
PCP	TCP	>1000	(b) (4)-25056 Yevich et al, 1990
Calcium Channel	Nitrendipine	>100000	(b) (4)-09455 Yevich et al, 1990
Sigma	(+)N-allylnormetazocine	1300	(b) (4)-25057 Yevich et al, 1990
Sigma	(+)3-PPP	710	(b) (4)-25079 Yevich et al, 1990

Gepirone is metabolized to at least 2 major metabolites in humans and these metabolites show moderate binding affinities at some or the receptors examined (see table, below).

Table 2. Summary of the Sponsor's summary data on affinities of gepirone and 2 major human metabolites (3'-OH-gepirone and 1-PP) at various receptors. Values represent IC50 and/or Ki values (nM). Hyphen (-) indicates that no data was provided. I have shaded the receptors for which gepirone and/or metabolites appear to have at least moderate affinity.

RECEPTOR	SUBTYPE (LIGAND)	GEPIRONE	3'-OH-GEP	1-PP
5-HT1	(5-HT)	3800	-	20,000
	1A (8OHDPAT)	10-100	-	≥1000
	1A-human clone	38, 97	58	420, 650
	1B-human clone	>7000	-	25,000
	1C-human clone	5000	-	-
	1D-human clone	>100,000	-	-
5-HT2	(spiperone)	≥3000	-	>10,000
	2A-human clone	>6000	2000	>55,000
	2C-human clone	>3000	>89,000	>89,000
5-HT6		>50,000	>55,000	>55,000
5-HT7		635	1800	9200
D1	(SCH 23390)	>1000	-	-
D2	(spiperone)	>1000	-	>10,000
	2-rat clone	58	-	-
	2L-human clone	900	12,000	>59,000
	2S-human clone	2600	18,000	>59,000
	3-human clone	120	1400	15,000
	4-human clone	190	420	>31,000
Alpha1	(WB-4101)	480-2400	-	≥10,000
Alpha2	(Clonidine)	>1000	-	20-50
	2A-human clone	3800	4500	85, 120, 300
	2B-human clone	13,000	20,000	350
	2C-human clone	1000	1500	610
Beta	(DHAlpren)	>100,000	-	≥2000
Muscarinic	(QNB)	>21,000	-	-
	M1-human clone	29,000	>46,000	>46,000
	M2-human clone	>10,000	>54,000	>54,000
	M3-human clone	>43,000	>43,000	>43,000
	M4-human clone	>51,000	>51,000	>51,000
Benzodiazepine		>1000	-	>10,000
GABA	(GABA)	>1000	-	>1000
	A (muscimol)	>1000	-	>100
Opioid	(U-69-593 or Nx)	>1000	-	-
CCK	(propiolyl-CCK5)	>100,000	-	>1000
IMI		>1000	-	-
H1	(pyrilamine)	>1000	-	-
Strych-Gly		>100,000	-	-
Kainate		10,000	-	-
NMDA	CPP	>100,000	-	-
AMPA		>100,000	-	-
PCP(MK-801)	(MK or TCP)	>1000	-	>100,000
Ca-antagonist	nitrendipine	>100,000	-	-
Sigma		710,1300	-	>10,000

Conclusions from binding data: Gepirone appears to be selective for 5-HT_{1A} receptors (vs other 5-HT receptors and other receptors that were tested), with possible activity at 5-HT_7 receptors and some D_2-type dopamine receptors (viz., D_3 and D_4). 3'-OH-gepirone has similar affinity at 5-HT_{1A} receptor, with no significant affinity at 5-HT_2 receptors (at least at 5-HT2A and 2C), but it's affinity was not determined at other 5-HT1 receptor subtypes; it also appears to have some low affinity for D_4 dopamine D_2-type receptors. 1-PP appears to have moderate affinity for $alpha_2$ adrenergic receptors. None of these forms of gepirone seem to have affinity at muscarinic cholinergic receptors. Binding at other receptors cannot be commented on since it was not characterized for all 3 gepirone forms.

Affinities of gepirone and 3'-OH-gepirone for cloned human 5-HT_{1A} receptors were similar, with Ki of 38 and 58 nM, respectively ((b) (4) , NL0025880).

Questions from the binding data:

- Are gepirone and 3'-OH-gepirone agonists or antagonists at 5-HT_{1A} receptors? Both appear to be agonists; partial vs full agonism is not clear.

- Does 3'-OH-gepirone have anti-depressant potential in animal screening tests? Although gepirone was put through a complete screen, I only found 2 *in vivo* tests for 3'-OH-gepirone. Both geprirone and 3'-OH-geppirone increased reinforcement and decreased responding on DRL 72 test in rats, consistent with antidepressant activity. Both also increased latency to REM sleep and reduction of REM sleep in an automated classification of sleep organization test in rats, consistent with antidepressant with anxiolytic properties.

- Are geprione and 3'-OH-gepirone active at D_2-type receptors? The Sponsor agrees that gepirone exhibits some weak dopamine agonist activity (Wilderman, 1983; Nash and Meltzer, 1989).

- Is 1-PP active at alpha2 adrenergic receptors? Agonist or antagonist? Antagonist, blocks clonidine-elicited effects (e.g., hypothermia, hypolocomotion, slowing of GI motility, prolongation of hexabarb-induced loss of righting reflex).

NB Buspirone is labeled as having <u>high</u> affinity for 5-HT_{1A} receptors. Looking at the studies sited in the Sponsor's summary table below), shows that gepirone tends to have slightly lower (mean ~3-fold) afffinity for 5-HT_{1A} receptors than buspirone does; and both appear to have considerably lower affinity than 8OHDPAT (buspirone is 14- to 24-fold lower affinity than 8OHDPAT in the 3 studies below). Gepirone does not seem to qualify as high affinity binding with mean Ki = 40 nM (range = 13-70 nM in the 3 studies below). Buspirone also does not seem to have high affinity for 5-HT_{1A} receptors with mean Ki = 14 nM (range 9.2-20).

Table 3. Summary table showing relative affinities of gepirone and buspirone at 5-HT_{1A} receptors; tablulated from studies cited by the Sponsor.

STUDY	RECEPTOR SOURCE (NON-SPECIFIC)	KI IC50	GEPIRONE	BUSPIRONE	GEP:BUS	8OHDPAT
(b) (4) NL0025880	Cloned human receptor (NS not specified)	Ki	38 nM	12 nM	3	0.5 nM
Piercy, et al, 1994	Cloned human receptor (NS excess 8OHDPAT)	Ki	13 nM	9.2 nM	1.4	0.5 nM
Hamik, et al., 1988	Rat (or bovine?) brain (NS excess 5-HT)	Ki	70	20	3.5	nd
Hanmon, et al., 1988	Rat hippocampus (NS 5-HT)	IC50	256 nM	45 nM	6 nM	3.3 nM
(b) (4) -11156	Rat brain (NS none specified)	IC50	54 nM	19 nM	2.8	nd

Pharmacology conclusions:

Labeling issues/Labeling that I would recommend:

Pharmacodynamics: The mechanism of action of Gepirone, as with other antidepressants, is unknown.

Preclinical studies indicate that gepirone binds selectively, with moderate affinity, to serotonin 5-HT_{1A} receptors. Major human metabolites of gepirone also exhibit *in vitro* binding activity: 3'-OH-gepirone binds with moderate affinity to 5-HT_{1A} receptors and 1-PP binds with moderate affinity to adrenergic alpha2 receptors. Neither gepirone nor its 2 major metabolites show significant affinity for muscarinic cholinergic receptors *in vitro.*

- The Sponsor asserts in labeling that "(b) (4) "

This detail regarding the exact mechanism of gepirone is speculative.

- The Sponsor asserts in labeling that gepirone has (b) (4) "

The evidence from the studies supporting these claims is weak and does not take into consideration the binding of major metabolites of gepirone. 3'-OH-geprione has moderate affinity for 5-HT_{1A} receptors similar to that of geprione. 1-PP binds with moderate affinity to alpha2 adrenergic receptors. None of the 3 seem to have significant affinity for muscarinic cholinergic receptors; and the human side-effect profile does not suggest muscarinic activity.

According to Dr. T.A. Hammad's Safety Review (2/26/02), the common adverse events with "incidence of 5% or more and a risk in the gepirone group twice or more the risk in the placebo group includes dizziness, nausea, insomnia, paresthesia, and vomiting."

II. SAFETY PHARMACOLOGY:

Only selected safety studies are briefly summarized here; most were reviewed in the original IND 23,952.

Neurological effects: Gepirone was active in animal models that are predictive of antidepressant and anxiolytic activity in humans. Convulsions were occasionally noted in some general toxicology studies, and gepirone lowered seizure thresholds for strychinine and picrotoxin in rats.

Cardiovascular effects: Intravenous administration of gepirone at doses from 0.1 up to 30 mg/kg to anesthetized dogs, with thioridazine as positive control (study accession no. (b) (4)-09697): fall in mean arterial pressure (~50mm Hg at doses ≥3 mg/kg) with ED30 of 0.27 mg/kg iv.; decreased heart rate at 10 and 30 mg/kg.

In another study (accession no. (b) (4)-20714), oral doses of 15, 60, and 200 mg/kg decreased (dose-related) systemic arterial pressure and heart rate in conscious rats. IP administration at 2.5 and 20 mg/kg to anesthetized dogs inhibited cardiac responses to several autonomic tests (e.g., responses to carotid occlusion and administration of norepinephrine or acetylcholine. At these same doses, gepirone decreased heart rate, systemic pressure, left ventricle systolic pressure (but not end diastolic pressure), contractility parameters (decreased P'max, P'max/P, P'/Pd40), and decreased cardiac output, with no effect on pulmonary artery pressure or EKG parameters.

1-PP by oral administration was tested in conscious rats (study accession no. (b) (4)-20281); 50 mg/kg (but not 15 mg/kg) decreased diastolic and mean arterial blood pressures, with no effect on heart rate.

Pulmonary effects: No formal study, however, increased respiration was noted in some general toxicology studies in dogs. Increased incidence of histiocytosis was noted in lungs of rats and dogs in general toxicology studies.

Renal effects: 30 mg/kg by oral gavage produced significant diuresis/natriuresis in volume-loaded Sprague-Dawley rats (study accession no. (b) (4)-09622). Distended bladders were also noted in some toxicology studies.

Gastrointestinal effects: There was no GI toxicity in a 14-day study in Beagle dogs (3/sex/group; ~8kg body weights) testing extended-release tablets (20 mg; lot no. 98-013T) in daily doses of 20 mg or 100 mg (5 tablets) or placebo (5 placebo tablets) (Study accession no. (b) (4) 3469). Necropsy exam included external surfaces of the body and all viscera; the following organs were analyzed for histopathology: cecum, colon, duodenum, esophagus, GI tract abnormalities, ileum, jejunum, rectum, stomach, and gross lesions. All dogs survived, with no overt clinical signs of toxicity. However, body weights were decreased in HD females during days 1-4 of dosing and food consumption (400 g was provided daily, overnight; on average dogs ate ~240 g/day (females) or ~270 g/day (males)) was increased in HD males during days 1-8. At necropsy there were no treatment-related lesions. The only GI lesion observed was minimal hemorrhage in the submucosal lymphoid follicles of the cecum, colon, and rectum, at similar

incidence in controls and drug-treated dogs, and noted as probably agonal by the pathologist. There were no signs of GI irritation at either dose of gepirone.

Abuse liability: Gepirone did not show abuse potential in several animal tests. It was not self-administered by Rhesus monkeys that had been trained on cocaine. It did not substitute for diazepam, amphetamine or cocaine in drug discrimination paradigms in rats. It only weakly substituted for LSD, but blocked responding for LSD. Gepirone (up to 1 mg/kg iv acutely or 0.1 mg/kg daily for 10 days) did not alter responding for cocaine in Rhesus monkeys. Repeated dosing with gepirone (20 mg/kg po for 5 days) did not alter the acute LD50 for ip cocaine HCl and lowered the LD50 for ip morphine sulfate in rats (from 938 mg/kg to 166 mg/kg). It is not clear whether there are withdrawal signs when repeated geprione dosing is terminated in animals.

Dependence to gepirone was not directly addressed, however, the recovery arms of the chronic toxicology studies could offer some information regarding withdrawal signs, such as weight loss, when dosing was discontinued. In a toxicity study in dogs, the Sponsor concluded that there was no evidence of withdrawal signs during 3 drug-free months after 1-year of daily dosing in dogs. However, there was some evidence of increased incidence of diarrhea in dogs that had received the high dose (16 mg/kg/d). In a study in rats (3-month drug-free recovery after 6 months of daily, dietary dosing), there was no evidence of decreased body weights or food consumption, but the earliest time assessed after termination of dosing was 2 weeks, after withdrawal signs would be expected to be finished.

[NB (b) (4) submitted animal studies (largely from published literature) suggest that gepirone was not self-administered, not perceived as any of several drugs with abuse potential, namely diazepam, amphetamine or cocaine, and blocked recognition of LSD.

However, potential development of dependence on gepirone may not have been adequately investigated. The Sponsor concluded that there was no evidence of withdrawal signs during 3 drug-free months after 1-year of daily dosing in dogs; however, there is some evidence of increased incidence of diarrhea in dogs that had received the high dose (16 mg/kg/d). In a study in rats (3-month drug-free recovery after 6 months of daily, dietary dosing), there was no evidence of decreased body weights of food consumption, but the earliest time assessed after termination of dosing was 2 weeks, after withdrawal signs would be expected to be finished.

In a drug-discrimination study, gepirone was weakly perceived as LSD, which suggests possible abuse potential. LSD (and other hallucinogens) are not reliably self-administered

Additionally, repeated dosing with gepirone potentiated the lethality (i.e., decreased the LD50) of morphine, but not that of cocaine. It is not clear whether this effect could have impact for abuse liability of gepirone.]

Safety pharmacology summary and conclusions: Gepirone is a centrally acting drug and was active in animal models that are predictive of antidepressant and anxiolytic activities in humans.

Convulsions were occasionally noted in some general toxicology studies, and gepirone lowered seizure thresholds for strychinine and picrotoxin in rats. Acute cardiac effects were reversible and included decreased heart rate, mean arterial pressure, and contractility, with no apparent alterations in EKG parameters. Gepirone has potential for diuretic activity, in view of the increased diuresis and natriuresis noted for rats. Animal studies did not indicate abuse liability for gepirone; the slight generalization to LSD in an animal drug discrimination paradigm would have been noted as hallucinations in human subjects in the clinical trials, if it were meaningful. No major safety issues were raised by the animal safety studies that could not be monitored in humans.

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

In humans, oral bioavailability of gepirone was low (F=14-17%); dosing with a high fat meal increased the systemic exposure to gepirone (62% increase in C_{max} and 24% increase in AUC; at 20 mg ER dose). The terminal half-life for gepirone was 2.5-3 hr (IR formulation). Systemic exposure to geprione was attributed to parent drug and 2 major metabolites, 3'-OH-geprione and 1-PP, both present in plasma at higher levels than unchanged gepirone. [NB When I looked at the details of the ^{14}C-gepirone mass-balance study (accession no. CN105-007), gepirone accounted for ~2% and 1-PP accounted for ~7% of the total radioactivity (comparing $AUC_{0-\infty}$, estimated by the Sponsor from samples collected from 0.5 to 24 hr after dosing); 3'-OH-geprione was not measured in this study. In other studies where 3'-OH-gepirone was measured, it represented approximately 4-times the level of circulating gepirone and approximately 2-times the level of circulating 1-PP, or ~8-14% of total circulating radioactivity from gepirone. This indicates that these 3 compounds would account for only ~20% of the total circulating gepirone-derived radioactivity in the mass-balance study.] Metabolism was mediated by CYP3A4 and to a lesser extent by 2D6. The terminal half-life for gepirone was 2.5-3 hr (IR formulation). Elimination was predominantly in urine (81% of administered dose, compared with 13% in feces). [Summarized from Dr. G. Fetterly's Clinical Pharmacology and Biopharmaceutics Review of this NDA, dated 2/19/02, except where otherwise noted.]

The Sponsor reanalyzed TK data from several studies in rats and dogs to "generate missing values for both AUC and Cmax values, which were not always calculated in the original studies," and presented the results in study no. (b) (4),NL 0023418 (report dated September 2000). Below I have presented the results from the 3-month study in rats and the 1-year study in dogs. Only exposure verification, not kinetic analysis, was performed on longer studies in rats (see summary of 6-mo study results, below, and reviews of 12-mo general toxicity study and 2-yr carcinogenicity study, in their respective sections below).

Three-week oral range finding study and three month subacute toxicity study of MJ13805-1 **in rats** (study accession no (b) (4)-10108). Only parent drug was quantified and plasma levels were very variable in both these studies and only reliably measurable after a single dose in MD and HD females and after 3 months of dosing in all groups (see graphs below). From these curves, it is not clear what the PK/TK parameters are, however, the Sponsor calculated AUC0-6h, Cmax, and Tmax and these values are presented in the table, below.

Figure 1. Plasma curves for gepirone in male and female rats after 3 months of daily oral gavage dosing. [Graphed from Sponsor's mean values presented in study no. (b) (4) ,NL 0023418.]

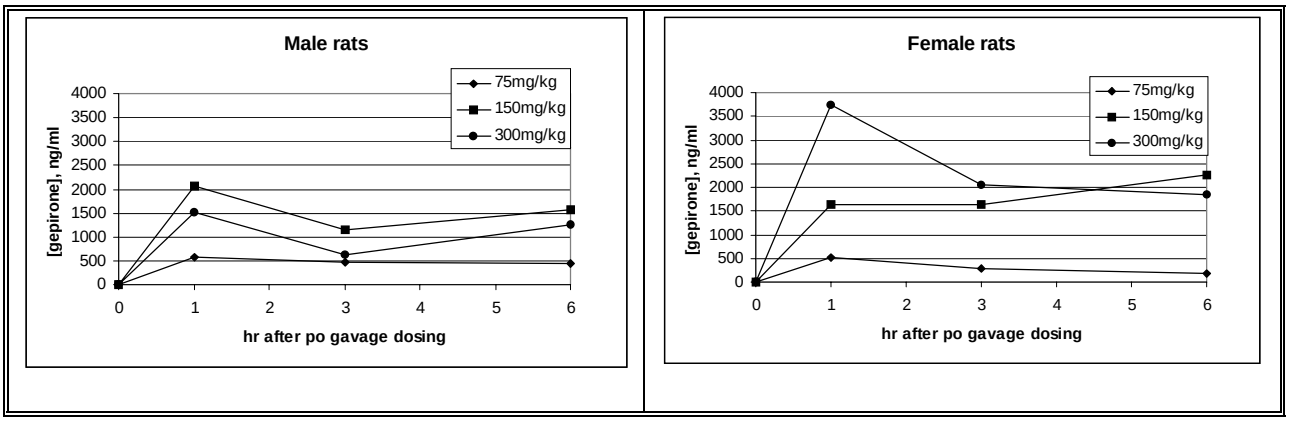


Table 4. Sponsor's tables showing PK/TK parameters calculated from the plasma curves, presented in the figure above, from the 3-month rat toxicity study. *Caveat emptor*.

Females mean values

Dose (mg/kg) Regimen	75 SD@	75 MD	150 SD	150 MD	300 SD$	300 MD
AUC(0-6) (ng.h/mL)	-	1756	934	9926	9395	13513
C_{max} (ng/mL)	-	528	193	2272	3133	3737
T_{max} (h)	-	1.0	1.0	6.0	6.0	1.0

Males mean values

Dose (mg/kg) Regimen	75 SD@	75 MD	150 SD@	150 MD	300 SD@	300 MD
AUC(0-6) (ng.h/mL)	-	2690	-	8331	-	5734
C_{max} (ng/mL)	-	575	-	2055	-	1510
T_{max} (h)	-	1	-	1.0	-	1.0

Mean plasma curves were constructed to calculate the toxicokinetic parameters; SD= single dose; MD= multiple dose

@ not calculated because plasma levels were not detectable or there were too few data points

Verification of exposure in 6-mo oral (dietary) study in rats (Study no. 13805-002-60-86; accession no. (b) (4) -20819): daily doses of 6, 12, or 24 mg/kg; blood sampled within 2 hrs of food removal at weeks 2, 10, and 26; presence of 1-PP verified in all dose groups (except LDM at weeks 2 and 10); presence of gepirone only in HD females at week 26; no clear dose-response; LOQ=10 ng/ml.

Verification of exposure in 12-mo oral ((b) (4) capsule) study in Beagle dogs (Study no. 13805-101-20-85; accession no. (b) (4) -20853): Daily doses of 0, 4, 8, or 16 mg/kg (5/sex/dose); blood samples drawn prior to dosing and 0.5, 1, 3, 6, and 24 hr after dosing at week

2, 11, 25, and 50; gepirone and 1-PP were quantified. There were no clear sex-differences in plasma levels; plasma levels of gepirone tended to decrease over the 52 weeks of the study, especially at the high dose; 1-PP levels remained consistent throughout the study. I have pooled values for males and females from the 52-week measurementss (see graphs below). It should be noted that it is not clear from these graphs that Cmax and Tmax have been reached at 6 hrs, so the exposures (Cmax and $AUC_{0\text{-}24h}$) cannot be accurately estimated from this data. Nonetheless, the Sponsor calculated PK/TK parameters, see table below.

Figure 2. Plasma curves for gepirone in Beagle dogs (5/sex, combined) after 1 yr of daily oral (capsule) dosing. [Graphed from Sponsor's mean values presented in study no. (b) (4) ,NL 0023418.]

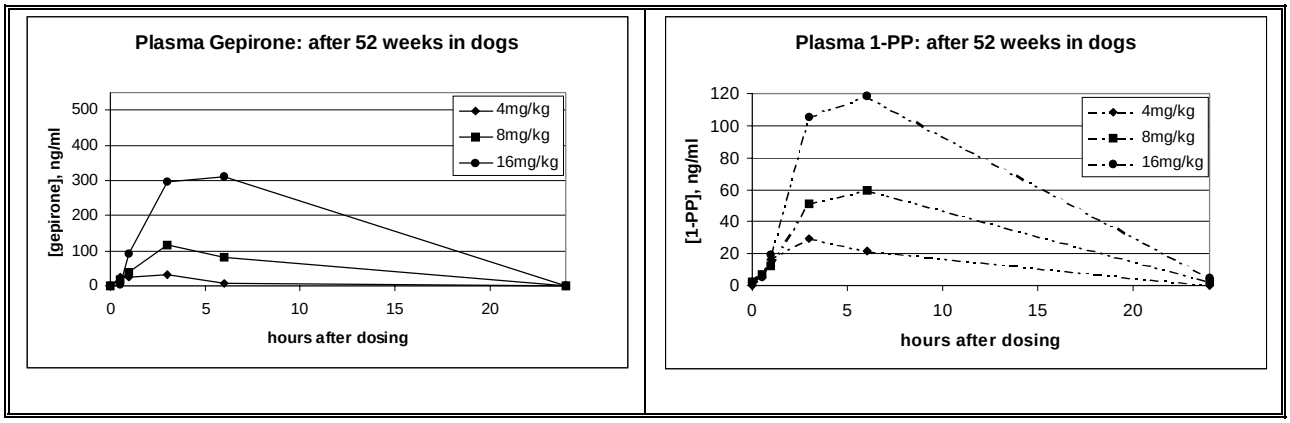


Table 5. Sponsor's tables showing PK/TK parameters calculated for gepirone and 1-PP from plasma curves, like those presented in the figure above, from the 1-year dog toxicity study. *Caveat emptor*. BEST AVAILABLE COPY

Gepirone levels in females

dose 4 mg/kg

Week number	2	11	25	52[a]
AUC(0-6) (ng.h/mL)	215 ± 144	176 ± 105	166 ± 101	192 ± 91
C_{max} (ng/mL)	81 ± 49	53 ± 29	61 ± 47	67 ± 27
T_{max} (h)	2.7 ± 2.2	3.6 ± 1.3	2.2 ± 1.1	1.5 ± 1.3

dose 8 mg/kg

Week number	2	11	25	52
AUC(0-6) (ng.h/mL)	628 ± 416	614 ± 336	704 ± 400	470 ± 313
C_{max} (ng/mL)	165 ± 120	193 ± 93	254 ± 142	148 ± 96
T_{max} (h)	4.2 ± 1.6	3.8 ± 2.2	2.1 ± 1.2	3.8 ± 2.2

dose 16 mg/kg

Week number	2	11	25	52
AUC(0-6) (ng.h/mL)	3450 ± 1002	1891 ± 454	2048 ± 865	1322 ± 320
C_{max} (ng/mL)	1068 ± 761	574 ± 123	664 ± 151	382 ± 71
T_{max} (h)	2.8 ± 2.0	4.8 ± 1.6	5.4 ± 1.3	4.8 ± 1.6

[a] n=3

Gepirone levels in males

dose 4 mg/kg

Week number	2[a]	11	25	52[b]
AUC(0-6) (ng.h/mL)	210 ± 32	204 ± 87	225 ± 63	164 ± 134
C_{max} (ng/mL)	62 ± 17	63 ± 29	71 ± 17	54 ± 53
T_{max} (h)	2.3 ± 1.2	4.8 ± 1.6	2.2 ± 1.1	4.0 ± 2.4

dose 8 mg/kg

Week number	2	11[c]	25	52
AUC(0-6) (ng.h/mL)	593 ± 449	491 ± 232	537 ± 289	384 ± 143
C_{max} (ng/mL)	187 ± 145	147 ± 54	164 ± 76	126 ± 35
T_{max} (h)	3.2 ± 1.8	5.3 ± 1.5	2.6 ± 0.9	4.8 ± 1.6

dose 16 mg/kg

Week number	2	11	25	52
AUC(0-6) (ng.h/mL)	1775 ± 522	1042 ± 473	1613 ± 715	651 ± 380
AUC(0-24) (ng.h/mL)	5093 ± 1607[b]	5299[d]	-	-
C_{max} (ng/mL)	442 ± 88	299 ± 128	450 ± 189	220 ± 165
T_{max} (h)	4.2 ± 1.6	4.8 ± 1.6	3.6 ± 1.3	5.0 ± 2.2

[a] n=2; [b] n=4; [c] n=2; [d] n=1; STD = standard deviation

1-PP levels in females

dose 4 mg/kg

Week number	2	11[a]	25	52
AUC(0-6) (ng.h/mL)	101 ± 35	84 ± 16	154 ± 53	129 ± 47
C_{max} (ng/mL)	28 ± 7.7	24 ± 3.0	39 ± 10	30 ± 7.4
T_{max} (h)	4.2 ± 1.6	4.5 ± 1.7	3.8 ± 2.2	3.0 ± 0.0

dose 8 mg/kg

Week number	2	11	25	52
AUC(0-6) (ng.h/mL)	181 ± 48	171 ± 85	191 ± 70	237 ± 82
AUC(0-24) (ng.h/mL)	-	-	-	866[c]
C_{max} (ng/mL)	50 ± 9.2	45 ± 15	50 ± 15	66 ± 12
T_{max} (h)	4.8 ± 1.6	4.2 ± 1.6	4.2 ± 1.6	4.8 ± 1.6

dose 16 mg/kg

Week number	2	11	25	52
AUC(0-6) (ng.h/mL)	510 ± 144	352 ± 52	417 ± 203	498 ± 89
AUC(0-24) (ng.h/mL)	1866 ± 57[b]	-	2769[d]	1611 ± 36[b]
C_{max} (ng/mL)	120 ± 26	106 ± 13	124 ± 52	128 ± 15
T_{max} (h)	3.8 ± 2.2	5.4 ± 1.3	5.4 ± 1.3	5.4 ± 1.3

[a] n=4; [b] n=2; [c] n=1

1-PP levels in males

dose 4 mg/kg

Week number	2[a]	11[b]	25	52[c]
AUC(0-6) (ng.h/mL)	93 ± 23	87 ± 24	154 ± 48	131 ± 78
C_{max} (ng/mL)	25 ± 6.4	24 ± 4.3	39 ± 11	35 ± 12
T_{max} (h)	3.0 ± 0.0	4.0 ± 1.7	3.0 ± 0.0	5.3 ± 1.5

dose 8 mg/kg

Week number	2	11[a]	25	52
AUC(0-6) (ng.h/mL)	150 ± 39	189 ± 98	220 ± 71	161 ± 46
C_{max} (ng/mL)	41 ± 8.0	57 ± 21	53 ± 15	56 ± 15
T_{max} (h)	4.8 ± 1.6	6.0 ± 0.0	3.6 ± 1.3	6.0 ± 0.0

dose 16 mg/kg

Week number	2	11	25	52
AUC(0-6) (ng.h/mL)	368 ± 96	299 ± 100	372 ± 118	321 ± 123
AUC(0-24) (ng.h/mL)	1485 ± 483[c]	1317 ± 371[c]	1649 ± 409[c]	1403 ± 407[b]
C_{max} (ng/mL)	91 ± 23.9	82 ± 26	103 ± 22	94 ± 33
T_{max} (h)	4.8 ± 1.6	6.0 ± 0.0	5.4 ± 1.3	6.0 ± 0.0

[a] n=3; [b] n=4; [c] n=2; [d] n=1; STD = standard deviation

Absorption: apparently not directly addressed.

Distribution: In male rats (Crl:CD(SD)BR; 3/time point; Study no. 854-MJ 13805-02; accession no. (b) (4) -12132), tissue distribution of total gepirone (parent plus metabolites) was determined at 1, 3, 6, 24, and 72 hr after a single, orally administered dose of gepirone (24 mg/kg; ^{14}C-labeled on pyrimidyl; after over-night fasting). Gepirone was present in several tissues at concentrations greater than in plasma, but by 72 hr after dosing, only kidney, liver, adrenals, and intestine still had measurable levels (see table, below).

Table 6. Sponsor's table showing tissue distribution of total radioactivity from orally administered ^{14}C-gepirone in male rats. Values represent mean ± standard deviation and are given as μg gepirone equivalents per g of tissue (or per ml of plasma).**

Tissue	Hours After Drug Administration				
	1	3	6	24	72
Plasma**	11.03 ± 1.17	6.19 ± 1.46	2.07 ± 0.98	0.13 ± 0.03	<0.04
Heart	13.10 ± 0.60	7.79 ± 1.69	2.68 ± 0.45	0.52 ± 0.08	<0.04
Lung	18.28 ± 1.68	9.54 ± 1.91	3.17 ± 1.02	0.28 ± 0.02	<0.04
Kidneys	54.91 ± 8.21	28.90 ± 5.50	10.33 ± 1.27	1.79 ± 0.04	0.54 ± 0.07
Liver	78.29 ± 6.80	52.31 ± 6.60	23.75 ± 1.84	8.38 ± 0.99	1.78 ± 0.12
Spleen	41.42 ± 1.30	20.75 ± 5.02	5.26 ± 1.79	0.49 ± 0.14	<0.04
Testes	13.07 ± 2.05	10.15 ± 1.43	3.06 ± 0.47	0.37 ± 0.01	<0.04
Brain	18.75 ± 0.68	9.57 ± 1.01	2.61 ± 0.71	0.11 ± 0.18	<0.04
Adrenals	17.14 ± 1.35	10.62 ± 4.13	4.08 ± 0.20	1.77 ± 0.43	0.45 ± 0.44
Stomach	107.40 ± 78.91	56.01 ± 5.63	12.03 ± 5.03	1.30 ± 0.93	<0.04
Intestine	193.61 ± 10.48	249.17 ± 20.50	254.65 ± 17.10	55.97 ± 4.20	0.35 ± 0.07
Fat	6.76 ± 1.75	3.55 ± 1.15	0.93 ± 0.14	<0.04	<0.04
Sk. Muscle	12.37 ± 0.94	6.89 ± 1.32	2.39 ± 0.40	0.23 ± 0.21	<0.04
Skin	13.04 ± 0.70	5.57 ± 0.41	2.31 ± 0.75	0.14 ± 0.24	<0.04

In a pilot study (accession no. (b) (4)-11198) using doses (lot 2B) of 10 and 75 mg/kg (14C-gepirone; by oral gavage, after 15-hr fasting) to rats, plasma and brain were assayed for gepirone and 1-PP as well as total radioactivity at 1, 3, 6, and 24 hr after dosing. After the 75 mg/kg dose, parent gepirone levels in plasma declined rapidly (I estimated a $t_{1/2}$ of ~2 hr) and represented a small part (<5%) of total radiolabel, while 1-PP declined more slowly (I estimated a $t_{1/2}$ of ~7 hr), in parallel with total radioactivity, and represented 20-25% of total radioactivity over the 24 hr period measured. 1-PP represented a larger fraction of radioactivity in brain (~50%) and gepirone sill accounted for only a small fraction of total radioactivity (<5%).

Maternal-fetal distribution of ^{14}C-gepirone in rats (Study accession no. (b) (4)-21572): single oral gavage dose of 6 mg/kg to pregnant rats (gestational day 18); sacrificed 1, 3, 6, or 24 hr later (3/time point); total radiolabel was determined for maternal plasma, heart, kidney, and liver, and for whole fetus and fetus heart and liver. The amount of radiolabel (gepirone-equivalents per gm) was essentially identical in maternal plasma and total fetus, at all time-points, and very similar for maternal and fetal hearts, whereas maternal liver levels were much (5-24-fold) greater than those in fetal liver. Geprione and/or metabolites crossed the placental barrier from mother to fetus.

Distribution to maternal milk of ^{14}C-gepirone in rats (Study no. 854-MJ13805-02; accession no. (b) (4)-11795): single oral gavage dose of 9.6 mg/kg to lactating rats (post-natal day 10-15); blood and milk samples 1, 2, and 4 hr later (5/time point); analyzed for total radioactivity, and gepirone and 1-PP. Gepirone plus 1-PP accounted for only ~10% of total radioactivity in maternal plasma and ~23% of that in milk. Although total radioactivity was lower in milk than in plasma at all time points (85% at 1 hr, 74% at 2 hr, and 59% at 4 hr), the amounts of geprione and 1-PP were 40-80% higher in milk than plasma (suggesting that levels of some other metabolite(s) were lower in milk than plasma).

Plasma protein binding: Plasma protein binding was determined *in vitro* for gepirone and 1-PP, a major metabolite circulating in human plasma; but not for 3'-OH-gepirone, the most abundant form of gepirone in human plasma (see table, below, from Study no. 817-89-259; accession no. (b) (4)-21085). In another study (accession no. (b) (4)-11762), similar binding (~65%) was seen for gepirone concentration between 25 and 125 ng/ml in human plasma.

Table 7. Sponsor's table showing *in vitro* plasma protein binding of geprione for humans, rats, dogs, and mice.

Mean Plasma Conc (ng/ml)	Percent Bound (SD)			
Gepirone	Mouse	Rat	Dog	Human
30.4	73.3 (0.9)	69.3 (11.7)	65.9 (2.7)	69.4 (7.7)
60.9	75.2 (1.7)	72.1 (8.4)	64.1 (1.2)	71.2 (5.6)
152.2	72.9 (2.1)	74.2 (3.2)	65.5 (6.7)	75.0 (5.5)
Mean (SD)	74.0 (1.8)	72.8 (3.0)	65.2 (3.7)	71.8 (6.0)
1-PP				
25.5	10.1 (0.1)	18.9 (2.5)	39.4 (7.1)	59.4 (7.3)
51.0	13.7 (2.6)	18.2 (1.1)	39.9 (6.0)	59.5 (4.9)
127.5	11.4 (3.0)	15.7 (3.2)	39.4 (6.3)	58.0 (5.2)
Mean (SD)	12.0 (2.6)	17.6 (2.6)	39.6 (5.6)	58.9 (5.2)

Metabolism:

In vivo metabolism in rats: When gepirone (10 mg/kg po; ^{14}C-labeled) was administered to rats (male Crl:CD(SD)BR VAF-PLUS rats: 3 for urine collection, 3 implanted with bile duct cannulas for bile collection), several metabolites, including 1-PP and 3'-OH-gepirone (the major metabolites circulating in humans), were found in 48-hr urine samples and 7-hr bile samples (see table, below; Study accession no. (b) (4)-25354). The metabolites in the table below account for ~80% of the radioactivity found in the 48-hr urine sample (68% of the dose was present in this urine sample) and ~60% of the radioactivity found in the 7-hr bile sample (51% of the dose was present in this bile sample).

Table 8. Sponsor's table of metabolites found in rat urine and bile after oral administration of ^{14}C-gepirone (10 mg/kg).

METABOLITE	PERCENT OF DOSE	
	URINE	BILE
1PP	24.4	0.9
5-OH-1PP	4.9*	3.5*
3'-OH-Gepirone	7.0	1.5
5-OH-Gepirone	2.4*	7.7*
3',5-di-OH-Gepirone	7.6	8.1*
5-OH-oxa-Gepirone	---	7.3*
tri-OH-Gepirones	7.2	3.1
unknown	4.8	1.6

* Primarily as glucuronide conjugate

In another study (accession no. (b) (4)-25355), male rats (2/time point) were treated with 10 mg/kg orally or 4 mg/kg intravenously and serum and brain samples were analyzed for total radioactivity, and gepirone and metabolites by HPLC. The major HPLC peaks in both brain and serum after iv or po were identified as 1-PP and 3'-OH-gepirone. Amounts of radioactivity in these 2 metabolite peaks, as well as that for gepirone and total radioactivity, are displayed in the Sponsor's table below. Both metabolites were present in brain and serum after administration by both routes. After oral administration of 10 mg/kg geprione, these 2 metabolites accounted for ~80% of the radioactivity in brain and ~60% of the radioactivity in serum; gepirone was only measurable at 1 hr and accounted for ~1% of the radioactivity in either serum or brain at that time point. 3'-OH-gepirone was present at approximately equal levels in brain and serum, however, 1-PP was ~3-times higher in brain at all time points, suggesting that it was actively transported into brain and/or synthesized there. Though not provided by the Sponsor, AUCs calculated from the serum curves (see table below) showed that 3'-OH-gepirone and 1-PP together account for ~50% of total radioactivity during the first 6 hr after oral dosing (with 10 mg/kg). Furthermore, exposures to 3'-OH-gepirone and 1-PP were 85-times and 180-times that for gepirone, respectively; much higher relative levels for these metabolites in rats than in humans.

Table 9. Sponsor's table showing mean concentrations of total radioactivity and of gepirone and metabolites in brain (ng/g) and serum (ng/ml) after oral and intravenous administration of ^{14}C-geprione to male rats (2/time point).

		Total ^{14}C		Gepirone		3'-OH-gepirone		1PP	
Route	Time (hr)	Brain	Serum	Brain	Serum	Brain	Serum	Brain	Serum
I.V.	1	1638	1231	352	322	258	326	892	273
(4 mg/kg)	3	607	337	8	*	34	40	327	138
	6	175	130	*	*	*	*	169	55
	24	*	12	*	*	*	*	*	*
P.O.	1	1860	1496	20	20	486	412	1133	489
(10 mg/kg)	3	1138	798	*	*	109	142	796	300
	6	878	523	*	*	63	73	662	221
	24	*	28	*	*	*	*	*	*

* Not detectable

Figure 3. Serum curves for total radioactivity, and gepirone metabolites 3'-OH-gepirone and 1-PP after a single oral dose of 10 mg/kg 14C-gepirone to male rats; left panel, arithmetic scale, right panel logarithmic scale (NB levels at 24 hr were not detectable (i.e., zero) for metabolites and do not appear on the log-graph on the right). [Graphed from Sponsor's values, see table above.]

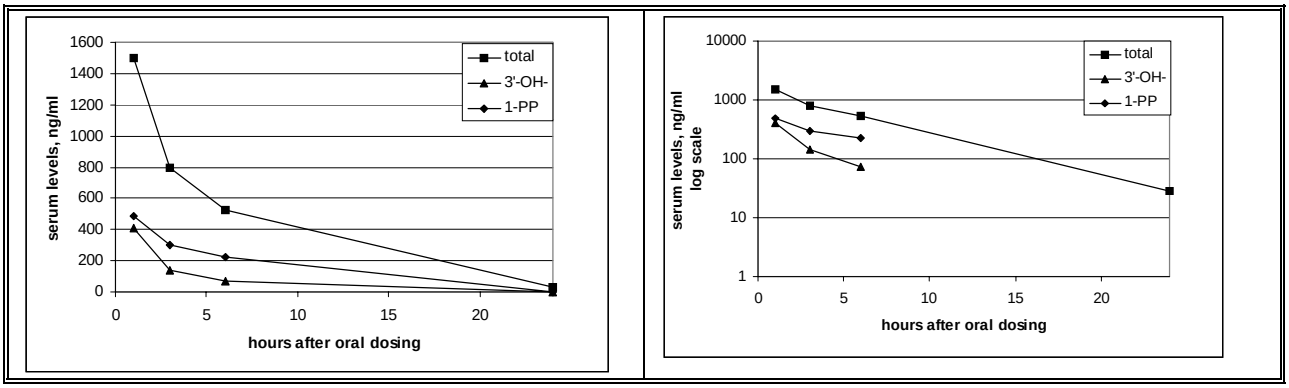


Table 10. AUCs calculated for total radioactivity, gepirone and 2 metabolites in serum after 10 mg/kg ^{14}C-gepirone po to male rats. [Calculated using trapazoid rule on Sponsor's mean values from table above.]

METABOLITE	$AUC_{0\text{-}6hr}$, ng.hr/ml	% OF TOTAL
Total radioactivity	5024	100%
gepirone	10	0.2%
3'-OH-gepirone	877	17%
1-PP	1816	36%

In (male) rats, both major human metabolites, 3'-OH-gepirone and 1-PP, were identified and quantified in serum and brain after oral administration of 10 mg/kg dose. Unchanged gepirone was scarcely measurable and represented less than 1% of total radioactivity in serum for 6 hr after dosing. 3'-OH-gepirone and 1-PP represented 17% and 36% of total radioactivity in serum for 6 hr after dosing. Both of these metabolites were also identified and quantified in urine and bile of rats following oral administration of gepirone.

In vivo metabolism in dogs: Metabolism was assessed in urine and plasma in (3) dogs for 48 hr after a single oral dose of (^{14}C-gepirone) 4.86 mg/kg (Study no. 904-13805-03; accession no. (b) (4) -50135). The amounts of parent and metabolites identified (see Table 11, below), accounted for ~80% of the total radioactivity recovered in urine in the 48-hr period (45.3% of the total dose was recovered in urine).

Table 11. Sponsor's table showing metabolites found in urine and plasma of rats after a single oral dose of ~5 mg/kg.

	URINE (% of Dose)	PLASMA (ng Equiv/ml)	
COMPOUNDS	0 - 48 hr	0 - 1 hr	2 - 4 hr
5-OH 1PP	*2.7	ND[a.]	ND
1PP	4.6	57	67
3',5-di-OH-gepirone	*7.4	*134	*228
3'-OH-gepirone	*4.9	*262	*180
5-OH-gepirone	*13.5	*507	*383
Gepirone	3.3	224	89

a. ND = None Detected
* Present as both free and conjugate.

Both major human metabolites, 3'-OH-gepirone and 1-PP, were found in plasma, as well as urine, of dogs in this study; 3'-OH-gepirone (free and conjugate) at higher and 1-PP at lower concentrations than gepirone for the 4 hr after dosing analyzed in this study.

In vitro metabolism: *In vitro* metabolism was investigated using S9 fraction from liver of mouse, rat, and dog (Study accession no. (b) (4) -25347); gepirone, as well as 6 potential metabolites (including 3'-OH-gepirone and 1-PP) were measured. The results are displayed in the table below. Notably, the major human metabolites, 3'-OH-gepirone and 1-PP, were made in all species. Both these metabolites have been verified *in vivo* for plasma of rats and dogs, but only 1-PP has been verified in plasma *in vivo* for mice (see verification of exposure data from the mouse carcinogenicity study).

Table 12. Sponsor's table showing gepirone metabolites (as % of initial amount of gepirone) following incubation of S9 fractions from liver of mice, rats, and dogs.

Metabolites	Mouse	Rat	Dog
5-OH 1-PP	0.3	2.5	0.0
1-PP	21.3	26.4	2.2
3,5-di-OH gepirone	13.9	11.5	1.5
3-OH gepirone	28.3	19.7	7.5
5-OH gepirone	5.5	7.6	27.6

Excretion: In (6) male rats (Crl:CD(SD)BR; Study no. 854-MJ 13805-02; accession no. (b) (4) -12132), 80% of a single, orally administered dose of gepirone (24 mg/kg; ^{14}C-labeled; after over-night fasting) was excreted (parent plus metabolites) within 24 hr (51% in urine, 28% in feces); 96% was recovered within 5 days (57% in urine, 39% in feces).

In a pilot study (accession no. (b) (4) -11198), similar results were obtained for doses of 10 and 75 mg/kg. At 10 mg/kg, 84% of the dose was recovered in the first 24 hr (49% in urine, 35% in feces); at 75 mg/kg, 67% was recovered in 24 hr (47% in urine, 20% in feces) and 87% was recovered in 72 hr (57% in urine, 30% in feces).

In rats, gepirone (parent plus metabolites) was largely excreted in urine (~60% of total dose), with the remainder in feces (30-40%).

PK/TK summary and conclusions: See overall summary in Detailed Conclusions and Recommendations section XI, below.

IV. GENERAL TOXICOLOGY:

A. Rats

1. *Summary of studies shorter than 1 year in duration*

The Sponsor submitted several acute, subacute and subchronic studies in rats:

- Acute toxicity of MJ 13805-1 [lot 4] [in ~6-week-old Crl:COBS CD(SD)BR rats] (Study no.13805-004-32-83, oral; 13805-005-32-83, ip; accession no. (b) (4)-10204):

The LD50 values for rats were 595 mg/kg orally and 208 mg/kg ip, with no evidence of sex difference. Hypoactivity and clonic-tonic convulsions (prior to death) were seen by both routes. The oral LD50 values for mouse, rat and rabbit were approximately equal. Necropsies were not performed.

- Subacute (two week) intravenous toxicity of BMY 13805-1 in [Crl:CD(SD)BR] rats (Study no. 13805-001-15-87; accession no. (b) (4)-12055; volume 38:129-385):

Daily dosing (from sterile stock solns batch E87H159, 1 mg/ml; batch E87H162, 2 mg/ml; batch E87H164, 4 mg/ml) at 0, 1, 2, or 4 mg/kg (10/sex/dose); no deaths; hypoactivity in all drug-treated rats; no remarkable effects on body weights or food consumption; no remarkabel changes in hematology or clinical chemistry; decreased liver weights in MD and HD males without histopathology.

- Three week oral rangefinding toxicity study of MJ 13805-1 [Crl:COBS CD(SD)BR] rats (Study no. 13805-001-11-83; accession no. (b) (4)/09867; volume 32:277-316):

Daily oral gavage doses (lot 4; in 0.5% methyl cellulose) of 0, 50, 100, 200, or 400 mg/kg (5/sex/dose); deaths not clearly drug-related; decreased body weight gains in males at ≥200 mg/kg/d, no clear effects on females; no effect on food consumption; at necropsy, no gross findings in controls, 200 or 400 mg/kg groups (specifically, no enlarged vessels of meninges noted). Sponsor concluded that doses for a future 13-week study should not exceed 300 mg/kg/d.

- A subacute four-week intramuscular toxicity study of MJ-13805-1 in [Crl:CD(SD)] rats (Study no. 631; accession no. (b) (4)-11653; volume 31:3-207):

Daily im doses (lot 15, ref no. E84G031; sterile stock soln lot 4703/1, 60 mg/ml) of 0, 15, 30, or 60 mg/kg (10/sex/group); no deaths; decreased spontaneous activity in most drug-treated rats; tremors frequently at HD; no remarkable effects on body weights; slight decrease in food consumption in MD and HD males; decreased total serum protein and/or albumin in males and MD and HD females was attributed to injection site inflammation; no remarkable effects on hematology, urinalysis; at necropsy, dose-related histopathological changes at injection site, “irritative lesions consisting of focal hemorrhage, neuritis, focal muscular necrosis, perimuscular

inflammatory cell infiltration and fibrosis, with associated reaction of iliac and popliteal lymph nodes."

- A subacute (3 month) oral [gavage] toxicity study of MJ 13805-1 in [Crl:COBS CD(SD)BR] rats (Study no. 13805-002-15-83; accession no. (b) (4)-10124):

Daily oral gavage doses (lot 5, in 0.5% methyl cellulose) of 0, 75, 150, or 300 mg/kg (10/sex/dose for toxicity). Several HD rats died during the study: 3 HD females (weeks 1 [intubation error], 4 [intubation error], 12 [enteritis, cecitis, colitis]) and 1 HD (week 9) male died [starvation due to dental malocclusion], 1 MD female was sacrificed with mass in left armpit (week 7). Hypoactivity was observed at MD and HD during week 1. Body weight gains were decreased in LD, MD and HD males, not in females; with no effects on food consumption. There were no remarkable effects on ophthalmoscopic exams, hematology; and only moderate increases in serum potassium and decreases in glucose in all treatment groups. At necropsy, relative adrenal weights were increased in LD, MD, and HD males, and HD females; liver weights were increased for all drug-treated females (absolute and relative) and MD and HD males (relative). There was dose-related pulmonary histiocytosis with accumulation of foamy macrophages in lung; increased mammary lobular development in females; dilatation of urinary bladder in a few females and most males. {NB At necropsy, no brain findings, gross or microscopic, specifically, no enlarged vessels of meninges noted.]

- A chronic (6 months) oral toxicity study of BMY 13805-1 in [~6-week-old Crl:CD(SD)BR] rats (Study no. 13805-001-20-86; accession no. (b) (4)-11925; volumes 35:3-352, 36:4-184).

Daily oral (dietary) dosing at 0, 6, 12, or 24 mg/kg (25/sex/group; necropsy on 15/sex/dose at week 27; remainder given 3-mo drug-free recovery period). There was no drug-related increase in mortality: only 2 rats died: 1 MDM found moribund at week 12 [starvation due to misalignment of incisors; maxilla also fractured]; 1 LDF found dead at week 11 [fractured maxilla]). Body weight gain was decreased 12% in HD males at week 13, vs controls; periodic increases in food consumption were observed in HD males and females. There were no remarkable effects on ophthalmology exams, clinical pathology, histopathology, or necropsy findings. [Pituitary weights were increased in MD and HD males; ovary weights were increased in HD females; both without histopathology.]

After drug withdrawal: ~10 rats per sex per dose were followed for 3 months after termination of dosing. Although there were no toxicities to assess for reversibility, this recovery leg of the study might give information on the dependence potential of gepirone; whether there are withdrawal symptoms, such as decreased body weight, etc., when gepirone administration was discontinued. There were no decreases noted for body weights or food consumption, however, body weights and food consumption were only measured every 2-3 weeks during "recovery" and the earliest measurement after termination of dosing seems to be 2 weeks after dosing was stopped (at week 28). Since the Sponsor does not directly address the issue of withdrawal symptoms, it is not clear that this study adequately addresses this issue.

2. *Study title: A chronic (one year) oral [dietary] toxicity study of BMY 13805-1 (gepirone) in rats.*

Key study findings:

- MTDs: 48 mg/kg/d for male rats and 16 mg/kg/d for female rats based upon decreased body weight gain at 13 weeks and decreased body weights throughout the 1-yr study.
- Slightly increased incidence of pulmonary histiocytosis in males and females at 48 mg/kg/d
- Increased incidence of distended bladders in males at 48 mg/kg/d
- Possible endocrine effects reflected in increased prostate and ovary weights and decreased uterus weights at 48 mg/kg/d.

Study no: 13805-002-20-87; accession no. (b) (4) -12527.
Volume #, and page #: volumes 39: 3-352 and 40:2-232.
Conducting laboratory and location: Bristol-Myers Co., Evansville, IN.
Date of study initiation: dosing initiated 11/11/87;
GLP compliance: yes, see volume 41:231-232.
QA report: yes, see volume 41:231-232.
Drug, lot #, radiolabel, and % purity: 2 lots were used: lot 18 (used for 1st 19 weeks) and lot 22 (Batch E88B175; (b) (4) batch no. 18 (b) (4) used for weeks 20 to termination).
Formulation/vehicle: admixture in Purina Rodent Laboratory Chow (Meal); prepared fresh weekly.

Methods:
Dosing:

Species/strain: male and female, Crl:CD(SD)BR rats (Charles River Breeding Labs, Portage, MI).
#/sex/group (main study): 22/sex/group.
Satellite groups used for toxicokinetics or recovery: none, but cited verification of exposure performed for the 2-year carcinogenicity study conducted concurrently with this study.
Age: ~6 weeks old at start of dosing.
Weight:
Housing: individually, in stainless steel wire bottom cages, with food and tap water *ad libitum*, 12-hr light:dark cycle (6am to 6pm).
Doses in administered units: 0, 4, 12 (increased to 16 at week 19), and 36 (increased to 48 at week 19) mg/kg/d.
Route, form, volume, and infusion rate: dietary admixture in food, available *ad libitum*.

Observations and times:

Clinical signs: daily for general appearance and viability; carefully at weekly weighing for overt toxicity, palpable masses.
Body weights: weekly during 1st 13 weeks, then monthly.
Food consumption: weekly during 1st 13 weeks, then monthly.

Ophthalmoscopy: prior to study initiation and during weeks 13, 27, and 52.
EKG: not done.
Hematology: non-fasted, at weeks 13, 27, and 52.
Clinical chemistry: and coagulation tests at necropsy (fasted) from 1st 10/sex/group.
Urinalysis: 18-hr, without food, at weeks 13, 27 and 51, from 1st 5/sex/group.
Gross pathology: all rats at termination and early decedents.
Organs weighed: see histopathology Table 15, below.
Histopathology: see histopathology Table 15, below.
Toxicokinetics: not done; Sponsor cited verification of exposure performed for the 2-year carcinogenicity study conducted concurrently with this study.
Other:

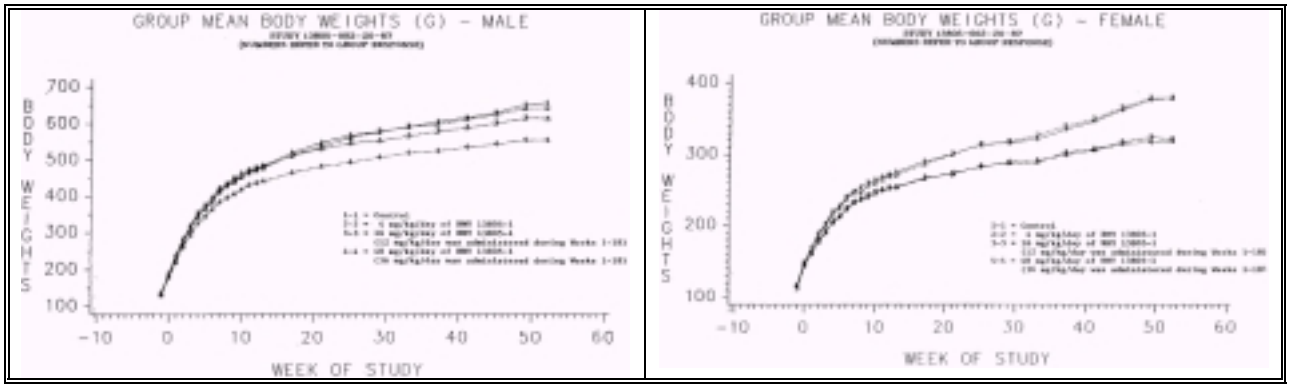
Results:

Mortality: Only 4 rats died (all found dead) during the study: 3 controls (1 male, #16 on day 48, of chronic nephritis; 2 females, #12 on day 31, accidentally (a cage fell on its head), #14 on day 28, of pyelonephritis) and 1 HDF (#16 on day 25, of accidental trauma (nose stuck in wire mesh of cage, an incisor broken).

Clinical signs: No remarkable findings; specifically, no mention of behavioral effects, like decreased activity.

Body weights: Body weight and body weight gain were decreased in MDF and HDM and HDF (see Figure 4, below). After 13 weeks of dosing (at the original doses), body weights (and body weight gain) were significantly decreased in MDF (BW ↓ 7%, BWG ↓ 11%) and HDF (BW ↓ 8%, BWG ↓ 14%) and HDM (BW ↓ 9%, BWG ↓ 14%) compared with controls. Body weights remained decreased through the end of the study (MDF ↓15%, HDF ↓ 16% and HDM ↓15%).

Figure 4. Gerirone decreases body weights (and body weight gain) in male and female rats. Sponsor's graphs of body weight data. Note that MD and HD were increased at week 20 of dosing (from 12 to 16 mg/kg/d and from 36 to 48 mg/kg/d, respectively).



Food consumption: Food consumption was increased in HDF. All males ate ~20-24 g/day throughout the study, with no differences between groups. Control, LD and MD females ate ~16-17 g/day, however, HDFs ate ~2 g/day more from week 6 throughout the study.

Drug intake: Closely matched nominal daily dosing throughout the study; based upon measured food consumption and nominal drug concentration in food.

Ophthalmoscopy: no remarkable effects.

Electrocardiography: not done.

Hematology: no remarkable effects at 13, 27, or 52 weeks.

Clinical chemistry: no dramatic effects. Several parameters were significantly altered, especially at HD, and probably reflect decreased body weights and food consumption (see Table 13, below).

Table 13. Dietary gepirone for 1 year slightly altered several clinical chemistry parameters in rats.

PARAMETER	SEX	LD	MD	HD
↓**Glucose**	M F	↓14%	↓12% ↓14% (NS)	↓19% ↓23%
↓**Total protein**	M F			 ↓10%
↓**Albumin**	M F			 ↓10%
↓**ALT**	M F			↓31% ↓46% [1]
↓**Total bilirubin**	M F		 ↓33%	 ↓33%
↓**Total cholesterol**	M F		↓24% (NS)	 ↓29%
↓**Triglycerides**	M F	↓43% ↓44%	↓33% (NS) ↓53%	↓37% (NS) ↓57%
↓**Uric acid**	M F			↓45% ↓24% (NS)
↑**Phosphate**	M F	 ↑23%	 ↑20%	 ↑29%
↓**Creatinine**	M F			 ↓10%
↑**Creatine kinase**	M F	 ↑75%	 ↑38% (NS)	 ↑48% (NS)
↓**Sodium**	M F			 ↓2%
↑**Potassium**	M F	 ↑12% (NS)	 ↑23%	 ↑20%

NS: not statistically significant by Dunnett's test (2-tailed, $p<0.05$).
[1]: 7/9 values were below the data range for controls; a single value was 5-times the mean of the other 9 values and > 2SDs above the mean for the group (including that value) and has been omitted as an outlier for this table.

Urinalysis: no remarkable effects noted; individual data, but not summary tables, provided.

Organ weights: HDM and HDF and MDF had decreased body weights and several organs whose absolute weights were decreased in these groups, normalized when adjusted for body weights. Exceptions were prostates in MDM (↓23% absolute, 16% relative) and HDM (↓29% absolute, 16% relative), with increased incidence of prostatitis at HD; and uteruses in HDF (↓33% absolute, 20% relative), with no attendant histopathology. Ovary weights were increased (60% absolute) in HDF, with no attendant histopathology.

Gross pathology: Bladders were distended in HD males (8/22), without attendant histopathology.

Histopathology: Drug-related findings were minimal and limited to **1)** slightly increased incidence of pulmonary histiocytosis (i.e., foamy macrophages in alveoli) at HD (minimal to slight in 4/22 HDM, minimal in 2/22 MDM vs 0/22 controls; minimal in 6/22 HDF vs 1/22 controls); and **2)** slightly increased incidence of prostatitis in HDM (minimal to moderate in 5/22 HDM vs moderate in 1/22 controls) that was considered spontaneous or incidental by the Pathologist. NB No abnormalities (e.g., no interstitial cell hyperplasia or adenomas in testes) were noted in other male sex organs (viz., testes, seminal vesicles, epididymis); and spermatogenesis was noted as moderate in all MD and HD males, slight to moderate in all LD males, and minimal to moderate in all control males.

Toxicokinetics: not done; the Sponsor cited verification of exposure performed for the 2-year carcinogenicity study conducted concurrently with this study (see table, below).

B. Dogs

1. *Summary of studies shorter than 1 year in duration*

The Sponsor submitted several acute, subacute and subchronic studies in dogs:

- Acute toxicity of BMY 13805-1 (gepirone) in [Beagle] dogs by oral administration (Study no. 13805-101-30-89; accession no. (b) (4)-12895; volume 36:323-351):

Single doses (lot 22, batch E88B175; capsule) of 15, 27, 42, and 45 mg/kg to males (1/dose) and 15, 19, 29, and 45 mg/kg to females (1/dose); no mortality; tremors and increased respiration, as well as salivation and emesis, were noted at most doses.

- A subacute two-week intravenous toxicity study in [Beagle] dogs with BMY-13805-1 (Study no. 640; accession no. (b) (4)-11922; volume 32:4-276):

15 daily iv doses (lot 13, batch E84D079; from sterile stock soln lot 4700/01, 12 mg/ml) of 0, 0.5, 1.0, and 2.0 mg/kg (3/sex/dose); no deaths; dose-related hypoactivity, tremors, ataxia and

increased respiration; no remarkable effects on food consumption, body weights, hematology, coagulation, serum chemistry, urinalysis, ophthalmological exam, EKG; no remarkable findings at necropsy.

- Three week oral rangefinding toxicity studies of MJ 13805-1 (rats and) dogs (Study no. 13805-101-11-83; accession no. (b) (4) /09867; volume 32:277-316):

Daily doses (lot 4; (b) (4) capsule) of 0, 3.75, 7.5, 15, or 30 mg/kg (1/sex/dose); ataxia, tremors, increased respiratory rate (panting), and protruding tongue frequent at HD, rare at lower doses; decreased body weights in all drug-treated females and all but LD male (vs slightly increased body weight in controls); HDF was anorectic for 3 days during week 1; chose 30 mg/kg/d for future 3-month study, because no life-threatening side effects at this dose. It's unclear why higher doses were not considered/used.

- A subacute four-week intramuscular toxicity study of MJ-13805-1 in Beagle dogs (Study no. 632; accession no. (b) (4) -11621; volume 30:86-322);

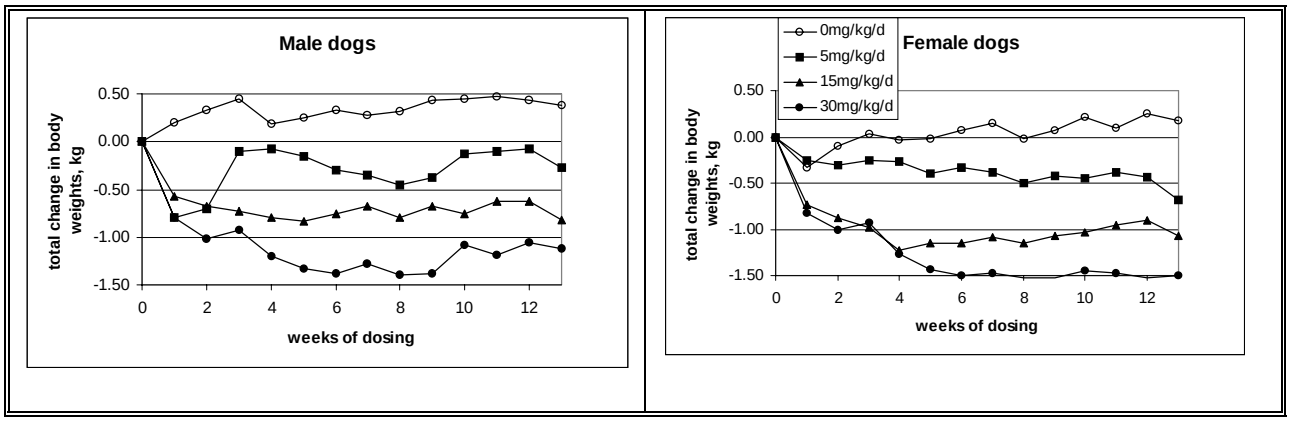
Daily im doses (lot 15, batch E84G031; from sterile stock solns lot 4700/01, 12 mg/ml; lot 4701/01, 6 mg/ml; lot 4702/01, 3 mg/ml) of 0, 1.5, 3, or 6 mg/kg (3/sex/dose); no deaths; dose-related frequency of hypoactivity, tremors, ataxia, and increased respiration rate; slightly decreased body weights at HD, no effect on food consumption; no remarkable effects on hematology, coagulation, serum chemistry, urinalysis, general physical exam, ophthalmological exam, EKG; no remarkable effects at necropsy, except that most drug-treated dogs showed subcutaneous and/or intramuscular red spots associated with a red color and/or size increase of popliteal and especially iliac lymph nodes, also found in some control dogs, but at lower intensity.

- A subacute (3 month) oral toxicity study of MJ 13805-1 in [~11-month-old] Beagle dogs (Study no. 13805-1-102-12-83; accession no. (b) (4) /10123; volume 33: 3-262).

Daily oral (capsule) doses (lot 4) of 0, 5, 15, or 30 mg/kg (3/sex/dose); feeding for 1 hr per day only; no deaths; tremors and increased respiration rates frequent in MD and HD groups; decreased weight gain, statistically significant through week 13 at HD, through week 10 for MDF and week 6 for MDM (see Figure 5, below); decreased food consumption in MD and HD females during week 1; no remarkable effects on hematology, coagulation, serum chemistry, urinalysis, general physical exam, ophthalmological exam, EKG; no remarkable effects at necropsy, except lower absolute and relative spleen weights in drug-treated females.

NB as in the 1-year study, the incidence of lens and corneal opacities seemed high in controls (at the end of the study, all control dogs had corneal opacities and 3/3 control females and 1/3 control males had lens opacities.

Figure 5. Gepirone (15 and 30 mg/kg/d po) decreased body weight gain in Beagle dogs. Graphic representation of data from Sponsor's table.



2. ***Study title: A chronic (12 month) oral [capsule] toxicity study of MJ 13805-1 in Beagle dogs.***

Key study findings:

- hypoactivity, tremors, hypertonia, increased respiratory rate, and salivation at MD and HD;
- emesis in all drug treated groups during week 1
- decreased body weights, dose-related, all doses (4, 8, and 16 mg/kg)
- decreased food consumption at 8 and 16 mg/kg
- increased erythrocyte sedimentation rates for males at 16 mg/kg throughout the study.

Study no: 13805-101-20-85; accession no. (b) (4) -11882.
Volume #, and page #: volumes 37:3-352, 38:3-128.
Conducting laboratory and location: Bristol-Myers Co., Evansville, IN.
Date of study initiation: 11/26/85, dosing was initiated; dosing was terminated on 11/25/86.
GLP compliance: yes, see volume 37, page 7 and volume 38, pages 127-128.
QA report: yes, see volume 38, pages 127-128.
Drug, lot #, and % purity: MJ 13805-1, lot no. 17, no purity information, only results of qualitative analyses were provided (EA, IR, NMR, MS).
Formulation/vehicle: ¼ oz. (b) (4) capsules, empty for control dogs, containing test substance for drug-treated dogs.

Methods:
Dosing:

Species/strain: male and female Beagle dogs ((b) (4)).
#/sex/group (main study): 5/sex/group were dosed for 12 months, with 3/sex/group necropsied after 12 months of dosing and the remaining 2/sex/group given a 3-month, drug-free recovery period before necropsy.

Satellite groups used for toxicokinetics or recovery: 2/sex/group for recovery.
Age: ~8 mo old at start of dosing.
Weight: 7.4-11.8 kg at start of dosing.
Housing: 1, 2, or 3 per masonry run; access to food (Purina Canine Diet #5006) for ~1 hr every morning (except for various dogs in MD and HD groups that were given 24-hr access to food from week ~2 to week 6 of dosing due to poor food consumption); *ab lib* water.
Doses in administered units: 0, 4, 8, and 16 mg/kg/day. Capsules were filled weekly and analyzed weekly for weeks 1-4 and monthly thereafter; all measured values were within 10% of nominal doses and the overall mean measured doses averaged 99.8-100.9%.
Route, form: oral, 1 capsule per day, ~30 min after feeding.

Observations and times:

Clinical signs: daily for mortality and overt toxicity; weekly at weighing for subtler signs.
Body weights: weekly.
Food consumption: daily (M-F).
Ophthalmoscopy: direct and indirect, as well as tonometry (intraocular pressure); once pretest and during dosing weeks 13, 30, 40, and 50.
EKG: once pretest and during dosing weeks 13, 30, 40, and 50.
Hematology: twice pretest and during dosing weeks 14, 27, 39, 51 (and 65 for recovery).
Clinical chemistry: twice pretest and during dosing weeks 14, 27, 39, 51 (and 65 for recovery).
Urinalysis: once pretest and during dosing weeks 26, 52 (and 65 for recovery).
Gross pathology: all dogs.
Organs weighed: see Histology Inventory in Table 15, below.
Histopathology: see Histology Inventory in Table 15, below.
Toxicokinetics: on blood drawn during weeks 2, 11, 25 and 50, prior to dosing and at 0.5, 1, 3, 6, and 24 hr after dosing; analyzed for parent and metabolite MJ 13653, results reported separately.
Other: liver cytochrome P450 activity in samples taken at necropsy.

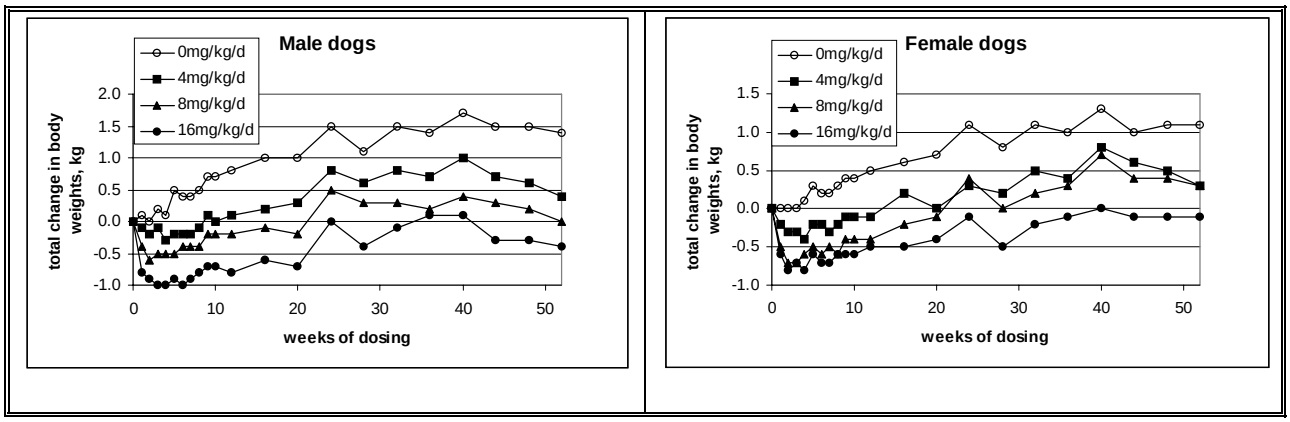
Results:

Mortality: No dogs died during this study.

Clinical signs: hypoactivity, tremors, hypertonia, increased respiratory rate, and salivation at MD and HD; emesis in all drug treated groups during week 1; At physical exams, areas of hair loss and/or reddened areas of skin were noted in some drug-treated dogs, but no controls.

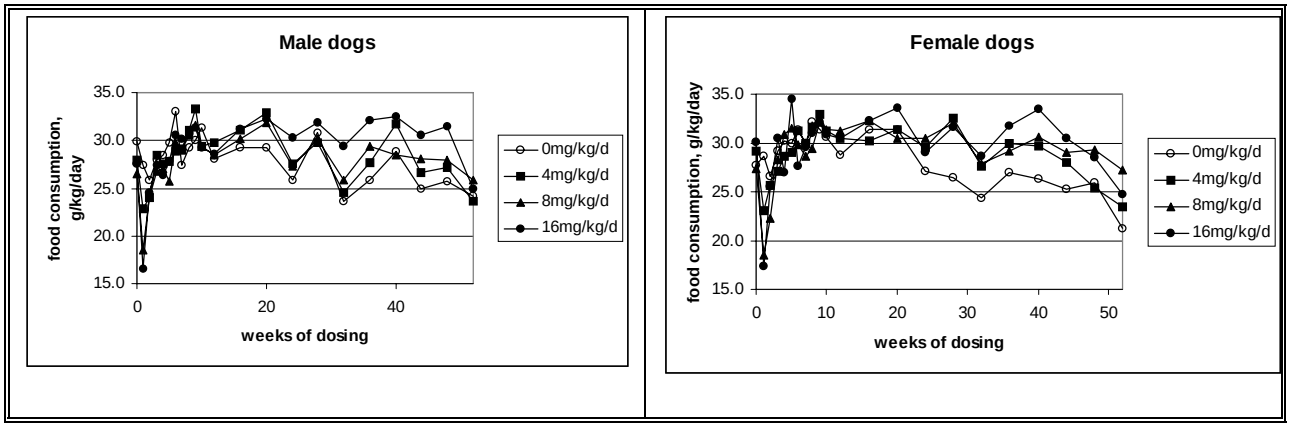
Body weights: Both MD and HD dogs (male and female) lost weight during the first week of dosing (mean ↓0.4g in MDM, ↓0.8g in HDM, vs ↓0.1g in LDM and ↑0.1g in male controls; mean ↓0.5g in MDF, ↓0.6g in HDF, vs ↓0.2g in LDF and no change in female controls). Based upon this decrease in body weights and the accompanying decreased food consumption, several dogs in the MD and HD groups were given 24-hr access to food in weeks 2-6. This strategy seems to have maintained food consumption, but body weights and body weight gains in these groups never matched the controls (see Figure 6, below).

Figure 6. Gepirone suppressed body weight gains in dogs. Graph of group mean values for change in body weights from week 0 (before dosing), from Sponsor's tabulated values. Values were provided for all 52 weeks of dosing, but only every 4th week (after week 12) is depicted below.



Food consumption: Food consumption was decreased in MD and HD dogs during the first week of dosing (see Figure 7, below). Mean consumption was only 18.5g/kg/d in MDM and 16.6g/kg/d in HDM compared with 26.5g and 27.6g, respectively, the week before dosing. Mean consumption was only 18.5g/kg/d in MDF and 17.3g/kg/d in HDF compared with 27.4g/kg/d and 30.1g/kg/d, respectively, the week before dosing. Dogs in MD and HD groups were given 24-hr access to food (rather than only 1-hr) during weeks 2-6 of dosing. This strategy seems to have allowed dosed dogs to consume at least as much food per day as controls throughout the rest of the study, however, body weights did not show the same return to control values (see above).

Figure 7. Gepirone initially suppressed food consumption in dogs. Graph of group mean food consumption, from Sponsor's tabulated values (g/kg/d); values were provided for all 52 weeks of dosing, but only every 4th week (after week 12) is depicted below.



Ophthalmoscopy: No effect of drug-treatment was evident in ophthalmological exams (e.g., corneal or lens opacities) or tonometry (intraocular pressure). There appeared to be a high

incidence of opacities of both lens and cornea in controls, with all control dogs having lens opacities and 3-4/5 per sex having corneal opacities by the end of dosing (week 50 test).

Electrocardiography: There were no apparent drug-related effects on respiration rate, heart rate or rectal temperature measured after 13, 30, 40, or 50 weeks of dosing. The Sponsor says that "mean values for EKG amplitudes, heart rate values, and intervals showed no apparent compound or dose related effects." Having looked at the individual data, I agree with the Sponsor. It is here noted that only QT_{abs} was provided; however, the lack of effect on heart rate, suggests that correction would not be likely to change the interpretation.

Hematology: Drug-related findings were limited to a 2-4-fold increased erythrocyte sedimentation rate in HD males in weeks 14, 27, 39 and 51.

Clinical chemistry: Drug-related findings were limited to slightly elevated glucose levels in HDM at 27 wks (↑16%) and 39 wks (↑12%). One MDM (#33) had elevated ALT (2-3-fold controls at weeks 14-51), AST (~ 2-fold controls at weeks 27, 35) and CK (3-4-fold controls at weeks 27-51), suggesting possible liver and/or cardiac toxicity, but without histopathological correlates.

Urinalysis: No remarkable findings.

Organ weights: No remarkable findings on either absolute or relative organ weights. Average terminal body weights for MD and HD females were 17% and 23% lower, respectively, than controls.

Gross pathology: There were no apparent drug- or dose-related findings at necropsy.

Histopathology: There were no apparent drug- or dose-related histopathology findings. In terms of reproductive potential, all males evidenced moderate spermatogenesis (2 sections of testes) and all females had follicular development, with primary, growing, and Graafian follicles, and corpora lutes (2 sections of ovaries). The MDM that had elevated ALT, AST, and CK did not show any evidence of pathology, specifically not in heart (3 sections) or liver, except for minimal multifocal lymphoid cell infiltration.

Toxicokinetics: Levels of gepirone and 1-PP (but not 3'-OH-gepirone) were quantified from blood samples drawn pre-dose and 0.5, 1, 3, 6, and 24 hr post-dose in weeks 2, 11, 25, and 50. Plasma levels of both gepirone and 1-PP tended to show dose-related increases, but the data was variable. Because most dogs showed peak levels at 6 hr after dosing, the Sponsor concluded that "…it is possible that the actual T_{max} might have been greater than 6 h." Consequently, the sampling points do not allow an adequate description of kinetic parameters. Despite these limitations, I have included the Sponsor's tables of mean C_{max} values for geprione and 1-PP in the table, below, as evidence of exposure to both parent drug and 1 major human metabolite (1-PP). (This data is also analyzed in the Pharmacokinetics Section of this review).

Table 14. Maximum plasma levels of gepirone (upper panel) and 1-PP (lower panel) determined within 6 hours of dosing in a 1-year study in dogs. [Sponsor's tables directly copied.]

Table 13. Mean± SD C_{max} Levels Of Gpirone (ng/mL) In Dogs (n=5/group) During A 1 Year Toxicology Study ((b) (4) .11882)

Week	Males			Females		
	4 mg/kg/day	8 mg/kg/day	16 mg/kg/day	4 mg/kg/day	8 mg/kg/day	16 mg/kg/day
2	65.9± 20.9	187.2±144.7	441.8±87.9	80.7±48.6	164.9±120.3	1067.9± 760.6
11	63.1±28.7	120.9±74.9	299.1±127.7	53.1±29.3	192.6±93.4	574.4±122.8
25	70.7±17.1	163.7±76.4	450.2±188.6	60.6±46.7	254.4±141.6	663.7±150.8
50	47.1±48.6	126.1±34.8	220.4±164.6	49.4±31.7	148.4±96.2	382.3±71.0

Table 14. Mean (SD) C_{max} Levels Of 1-PP (ng/mL) In Dogs (n=5/group) During A 1 Year Toxicology Study ((b) (4) .11882)

Week	Males			Females		
	4 mg/kg/day	8 mg/kg/day	16 mg/kg/day	4 mg/kg/day	8 mg/kg/day	16 mg/kg/day
2	24.8±5.6	41.1±8.0	91.3±23.9	28.0±7.7	50.1±9.2	120.2±25.8
11	21.3±6.2	48.1±26.3	82.4±25.9	23.4±3.3	44.9±15.5	105.6±12.8
25	39.2±11.0	52.9±15.4	103.3±21.8	38.7±9.8	49.9±15.4	124.4±52.4
50	31.2±13.9	56.5±15.5	93.8±33.3	29.5±7.4	66.2±11.5	128.4±15.2

After drug withdrawal: Two dogs per sex per dose were followed for 3 months after termination of dosing. Although there were no toxicities to assess for reversibility, this recovery leg of the study might give information on the dependence potential of gepirone as to whether there are withdrawal symptoms, such as decreased body weight, etc., when gepirone administration was interrupted. The Sponsor concludes that "… upon termination of MJ 13805-1 treatment, no remarkable clinical signs were observed, and dogs previously treated with MJ 13805-1 showed body weight gains comparable with control dogs." Since the Sponsor seems aware of the significance of withdrawal signs, I assume that dogs were adequately observed early after termination of dosing, however, body weight and food consumption data was only measured weekly. When I looked at the weekly incidence of clinical signs in these dogs, there was a dose-related increase in the fraction of weeks where diarrhea occurred during dosing, but also during the 3 months of gepirone withdrawal, especially in the HD group (2/2 HDM, 1/2 HDF and 1/2 MDF). This is not necessarily a specific measure for withdrawal and could reflect a protracted effect of gepirone.

Summary of individual study findings: See key findings at the beginning of this study.

Toxicology summary and conclusions: See overall summary in Detailed Conclusions And Recommendations section VIII.

Table 15. Histopathology Inventory for NDA 21-164.

Study	1-yr	1-yr	2-yr	2-yr
Species	Dog	Rat	rat	mouse
Adrenals	X*	X*	X	X
Aorta		X	X	X
Bone Marrow smear	X	X	X	X
Bone (femur)	X rib	-	X	X
Brain	X*	X*	X	X
Cecum	X	X	X	X
Cervix	X	-	-	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X*	X	X
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube	-	-	-	-
Gall bladder	X	-	-	X
Gross lesions	X	X	X	X
Harderian gland	-	-	-	-
Heart	X*	X*	X	X
Ileum	X	X	X	X
Injection site	N/a	N/a	N/a	N/a
Jejunum	X	X	X	X
Kidneys	X*	X*	X	X
Lachrymal gland	-	X	X	-
Larynx	X	-	-	-
Liver	X*	X*	X	X
Lungs	X	X	X	X
Lymph nodes, cervical	X	X	X	X
Lymph nodes mandibular	-	-	-	-
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity	-	-	-	-
Optic nerves	-		X	-
Ovaries	X*	X*	X	X
Pancreas	X	X	X	X
Parathyroid	X		X	X
Peripheral nerve	X	X	X	X
Pharynx	-	-	-	-
Pituitary	X*	X*	X	X
Prostate	X*	X*	X	X
Rectum	-	-	-	X

Study	1-yr	1-yr	2-yr	2-yr
Species	Dog	Rat	rat	mouse
Salivary gland	X	X	X	X
Sciatic nerve	-		?	X
Seminal vesicles	-	X	X	X
Skeletal muscle	X	X	X	X
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X	X
Sternum	-	X	X	X
Stomach	X	X	X	X
Testes	X*	X*	X	X
Thymus	X	X	X	X
Thyroid	X*	X*	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X	X
Vagina	X	X	X	X
Zymbal gland	-		-	-

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY:

A. Study title: Gepirone (BMY-13805): Ames microbial mutagenicity assay and Escherichia coli WP2 uvrA reverse mutation assay.

Key findings: Negative: Gepirone was not mutagenic, with or without metabolic activation, in an appropriate selection of bacterial tester strains.

Study no: Study #89006; accession no. (b) (4) -21057 (identification no. in electronic submission).
Study type: Ames test.
Volume #, and page #: volume 42, pages 0006-0044.
Conducting laboratory and location: Bristol-Myers Company, Thompson Road, Syracuse, NY.
Date of study initiation: full assay on 2/7/89; repeat assay (for strain TA98, because of technical problems in the initial test) on 2/22/89.
GLP compliance: yes, see page 44.
QA reports: yes, see page 44.
Drug, lot #, and % purity: BMY-13805-1, lot no. 22, .99.77% pure (by HPLC, C/A dated 11/14/88).

Formulation/vehicle: dissolved in Milli-Q water.

Methods:

Strains/species/cell line: Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537, and E.coli strain WP2 uvrA; adequate selection by current standards.
Dose selection criteria:

Basis of dose selection: 5 mg/plate as maximum dose; drug is soluble in water and higher doses had been previously tested in some strains, see below.
Range finding studies: study done in 1981 by (b) (4), in all the S. typhimurium strains used in the present study (plus TA1538), but not the E. coli strain; doses of 0.55, 1.7, 5.0, 15, and 45 mg/plate, with toxicity (i.e., decreasd revertants) evident for all but TA1537 at HD; test compound was not mutagenic with or without metabolic activation (NB TA98 with activation was just 2-fold control at 15 mg/plate, but essentially at control level at 5 mg/plate, and only 12% of control at 45 mg/plate).

Test agent stability: adequate: dosing solutions were frozen at –40 degrees C immediately after dosing, until analysis; assays ranged from 92-94% of nominal doses for full study and 95-95% for repeat study.
Metabolic activation system: S-9 fraction from livers of male Sprague–Dawley rats that had been treated with Aroclor 1254 was purchased from (b) (4), with protein concentration of 27 mg/ml (Lowry assay). MFO mix was made according to Maron and Ames (1983), so I assume it is adequate.
Controls:

Vehicle: Milli-Q water, DMSO for some positive controls.

Negative controls: vehicle.
Positive controls: 2-nitro-fluorene (TA98), 2-aminoanthracene (with metabolic activation for all strains), sodium azide (TA100 TA1535, 9-aminoacridine (TA1537), ENNG (E.coli WP2).

Exposure conditions:

Incubation and sampling times: plates were incubated for 48 h at 36-40 degrees C.
Doses used in definitive study: 0, 0.3125, 0.625, 1.25, 2.5, and 5.0 mg/plate.
Study design: (100 mm) plate incorporation assay.

Analysis:

No. of replicates: triplicates, except only duplicates for positive controls.
Counting method: bacterial lawns were evaluated under a dissecting scope; revertant colonies were counted with a New Brunswick Biotran II, Model C111 Electronic Colony Counter.

Criteria for positive results: quoting from the Sponsor's protocol:

"

A positive response to a test article is defined as follows:

1. A two-fold increase in the mean number of revertants per plate above the vehicle control in strains TA98 and TA100.

2. A three-fold increase in the mean number of revertants per plate above the vehicle control in strains TA1535, TA1537 and E. coli WP2 uvrA.

3. Increases in revertant counts for all strains must exhibit concentration dependence in order to warrant the designation of positive. That is to say, increasing concentrations of the test article must be accompanied by increasing numbers of revertants per plate.

4. A positive response in one tester strain either with or without exogenous metabolic activation is sufficient to designate the test article as a bacterial mutagen.

"

Summary of individual study findings:

Study validity: The study was valid.
Study outcome: Bacterial lawns were not affected in any strain, at any dose (except for a slight reduction noted for E. coli WP2 at the HD only). There was no indication of mutagenicity for gepirone in any strain tested, with or without metabolic activation.

B. Study title: BMY-13805-1: Chinese hamster ovary cytogenetic assay.

Key findings: Not adequate: negative for 5-hr treatment with gepirone, with or without metabolic activation. However, the study was not valid, because this negative finding should have been followed up with a study using continuous treatment with gepirone (without activation) for ~24 hr (1.5 doubling times) according to the current ICH Guidance, S2B Genotoxicity (1997).

Study no: study no. 89050; accession no. (b) (4)-21398 (identification no. in electronic submission).
Study type: *In vitro* chromosomal aberration test.
Volume #, and page #: volume 42, pages 161-204.
Conducting laboratory and location: Bristol-Myers Company, Thompson Road, Syracuse, NY.
Date of study initiation: 7/25/89 (not explicitly stated in the report, but deduced from data sheet for dosing solution verification; consistent with week projected in protocol and Q/A inspection dates).
GLP compliance: yes, see page 192.
QA reports: yes, see page 192.
Drug, lot #, and % purity: BMY-13805-1, lot no. 22, 99.77% pure (by HPLC, C/A dated 11/14/88).

Formulation/vehicle: Milli-Q water.

Methods:

Cell line: CHO-K1, an established Chinese hamster ovary fibroblast cell line, obtained from (b) (4), but cloned/grown in the current laboratory and designated CHO0K1-BR4.
Dose selection criteria:

Basis of dose selection:
Range finding studies: a preliminary study, using doses of 0.15, 0.30, 0.60, 0.80, and 1.0 mg/ml, duplicate cultures, except triplicates at HD, with and without metabolic activation, qualitative evaluation of metaphases indicated no drug effects, except slight cytotoxicity at HD with activation.

Test agent stability: adequate: dosing solutions were frozen at –20 degrees C immediately after dosing, until analysis; assays ranged from 96-99% of nominal doses.
Metabolic activation system:
Controls:

Vehicle: Milli-Q water.
Negative controls: vehicle.
Positive controls: mitomycin C (without metabolic activation) and cyclophosphamide (with activation).
Comments:

Exposure conditions:

Incubation and sampling times: cells were incubated with drug with or without S9 for 5h, then washed, and further incubated with fresh medium for ~19 hr, then treated with colcemid for 3 hours before metaphase spreads were prepared.
Doses used in definitive study: 0.4, 0.6, 0.8, 1.0 mg/ml.

Analysis:

No. of replicates: triplicate flasks.
Counting method: at least 50 metaphases were analyzed per treatment flask (i.e., per replicate).

Criteria for positive results: quoting from the Sponsor's protocol:

"

A response to the test article will be deemed positive if the following criteria are met:

1. A concentration-dependent increase either in the percentage of damaged metaphases or in the number of aberrations per cell should occur in two consecutive concentration levels.

2. There is a statistically-significant increase either in the percentage of metaphases exhibiting chromosome damage or in the number of aberrations per cell with at least two consecutive concentrations. The historical control for the mean percentage of damaged cells is 5.4 + 2.3 (S.D.) and for the mean number of chromosome aberrations is $0.06\bar{2}$ + 0.033 (S.D.) over thirteen determinations. These two historical control values will be included as factors along with the concurrent experimental controls when assessing the potential clastogenicity of the test article.

"

Summary of individual study findings:

Study validity: **Not valid**, because the negative results obtained with 5 hr treatment with gepirone (without activation) should have been followed up with continuous treatment (for ~24 hr) without activation.
Study outcome: **Negative, for 5-hr treatment time.** There was no evidence of increased chromosomal aberrations in cells treated with gepirone (for 5 hr) with or without metabolic activation (see table, below). However, this should have been followed up with a study using continuous treatment with gepirone (without activation) for ~24 hr (1.5 doubling times).

Table 16. Five-hr treatment with gepirone did not increase incidence of aberrant CHO cells. Sponsor's summary tables of incidences of aberrant cells treated for 5 hr with gepirone alone (upper panel) or in the presence of metabolic activation (lower panel).

(Absence of S-9)

Summary of Chromosomal Aberration and Aberrant Cell Frequencies

Treatment Group	Dose (µg/ml)	Treatment Culture Flasks Evaluated	Total No. Metaphases Evaluated	Mean Percent Aberrant Cells/ Culture (± Sd) Flask[1]	Mean No. Aberrations/Cells[2]
BMY-13805-1	1000	3	150	0 ± 0	0
	800	3	150	.7 ± .6	.007
	600	3	150	2.0 ± .6	.02
	400	3	150	1.3 ± 1.2	.013
Mitomycin C[3]	.25	3	150	48.67 ± 11.1	-
Water	20 µl	3	150	.7 ± .6	.007

[1] Percent aberrant cells equals (number of metaphases with at least one structural aberration) divided by (total metaphases evaluated per flask) X 100.

[2] Total structural aberrations, excluding gaps, divided by total number of metaphases evaluated in all culture flasks per dose group. Not including metaphases with greater than 10 structural aberrations.

[3] Aberrations not completely enumerated due to occasional metaphases with large number of aberrations.

(Presence of S-9)

Summary of Chromosomal Aberration and Aberrant Cell Frequencies

Treatment Group	Dose (µg/ml)	Treatment Culture Flasks Evaluated	Total No. Metaphases Evaluated	Mean Percent Aberrant Cells/ Culture (± Sd) Flask[1]	Mean No. Aberrations/Cells[2]
BMY-13805-1	1000	3	150	1.3 ± .6	.01
	800	3	150	.7 ± .6	.01
	600	3	150	.7 ± .6	.007
	400	1	50	0 ± 0	0
Cyclophosphamide[3]	10	3	150	32.0 ± 10.0	-
Water	20 µl	3	150	2.0 ± 1.2	.02

[1] Percent aberrant cells equals (number of metaphases with at least one structural aberration) divided by (total metaphases evaluated per flask) X 100.

[2] Total structural aberrations, excluding gaps, divided by total number of metaphases evaluated in all culture flasks per dose group. Not including metaphases with greater than 10 structural aberrations.

[3] Aberrations not completely evaluated due to occasional metaphases with large number of aberrations.

C. Study title: Gepirone hydrochloride: *in vivo* cytogenics in rats.

Key findings: Negative, and adequate: only 1 dose and only a single administration was used, however, the dose was 1/3 –1/2 the acute oral LD50, both male and female rats were used, and 3 time points were analyzed (6, 24, and 48 hr after dosing).

Study no: study no. 84123; accession no. (b) (4)-20232 (identification no. in electronic submission).
Study type: *In vivo* chromosomal aberration test of rat bone marrow cells.
Volume #, and page #: volume 42, pages 69-117.
Conducting laboratory and location: Bristol-Myers Company, Thompson Road, Syracuse, NY.
Date of study initiation: 1/8/85, deduced from chemical stability report.
GLP compliance: yes, see pages 71 and 101.
QA reports: yes, see page 101.
Drug, lot #, and % purity: MJ13805-1, lot # 15; purity not specified, but assayed at 99.8% (8/14/84).

Formulation/vehicle: suspended in 0.5% methyl cellulose aqueous solution.

Methods:

Strains/species: 8-week old male and female Sprague-Dawley rats (Crl:CD(SD)BR, Charles River, Wilmington, MA).

Dose selection criteria:

Basis of dose selection: approximately half the minimal lethal dose (see below)..

Range finding studies: In a preliminary study, doses of 50, 100, 200, 300, 400, 600, and 1000 mg/kg were administered by oral gavage: limiting mortality in females at doses ≥ 300 mg/kg and in males at ≥ 400 mg/kg (see table below); possible drug related loss of morphological integrity of some chromosomes at 300 mg/kg (see table, below).

Table 17. Sponsor's "Table 1": Range-finding results with gepirone hydrochloride in rats.

Dose[a] (mg/kg)	Bone Marrow Cytotoxicity: No. Animals ♂	♀	Deaths[c] ♂	♀	Qualitative Evaluation of Metaphases from 24-Hour Group (Observations)	Mean Metaphase Index[f]	Mortality: No. Animals ♂	♀	Deaths[h] ♂	♀
1000	1	1	1	1	-	-	-	-	-	-
600	2	2	2	2	-	-	5	5	4	5
400	0	0	-	-	-	-	5	5	1	4
300	3	3	0	0	PDR[d]	2.5%	2	2	0	1
200	3	3	0	0	NDR[e]	3.6%	2	2	0	0
100	3	3	0	0	NDR	3.4%	2	2	0	0
50	3	3	0	0	NDR	ND[g]	2	2	0	0
0[b]	3	3	0	0	-	3.5%	2	2	0	0

[a] Nominal dose in terms of mg gepirone hydrochloride bulk/kg.

[b] Negative control (0.5% methyl cellulose in water).

[c] Animals dying within 24 hours after treatment.

[d] PDR = possible drug-related effect indicated by loss of morphological integrity of some chromosomes. Some chromosomes were contracted while chromosomes in other metaphases were highly elongated.

[e] NDR = no apparent drug-related effect. Chromosome morphology similar to control animals.

[f] Percent of metaphases equals (number of metaphases per 500 cells per animal) divided by (500) X 100. Mean metaphase index is the mean percent of metaphases.

[g] ND = not determined.

[h] Animals were observed two days following treatment and no additional deaths occurred.

Test agent stability: gepirone solution was assayed 1 day after preparation and use; 101% of nominal (20 mg/ml)) concentration.
Metabolic activation system: *in vivo*.
Controls: (all 10 ml/kg by oral gavage).
Vehicle: 0.5% methyl cellulose aqueous solution.
Negative controls: vehicle.
Positive controls: triethylene melamine (TEM), in aqueous solution.
Comments:
Exposure conditions:
Incubation and sampling times: rats were dosed by oral gavage and sacrificed 6, 24 or 48 hr later (colcemid was injected ip ~2 hr before sacrifice); rats dosed with positive control were only sacrificed at 24 hr.
Doses used in definitive study: 200 mg/kg po (10 ml/kg).
Study design: 6/sex/treatment time/dose; after sacrifice, marrow was removed from both femurs and metaphase spreads were prepared, slides were stained with 5% Gurr's R-66 Giesma (pH 6.8).
Analysis:
No. of replicates: 100 bone marrow metaphases/rat, 5 rats/sex/group for gepirone and vehicle; 50 metaphases/rat, 4 rats/sex for TEM positive controls.
Counting method: direct counting of aberrant chromosomes; manual; microscopic.
Criteria for positive results: Not clearly specified. The Sponsor's protocol says,

"

A statistical evaluation may be performed to determine significance after each measured parameter has been examined by exploratory data analysis

methods to determine the nature and distribution of the data in order to select the most appropriate method for analysis."

Summary of individual study findings:

Study validity: Valid: only 1 dose and only a single administration was used, however, the dose was 1/3 –1/2 the acute oral LD50, both male and female rats were used, and 3 time points were analyzed (6, 24, and 48 hr after dosing).

Study outcome: Negative. There was no indication of increased frequency of aberrant cells or increased frequency of aberrations per cell due to gepirone treatment at 6, 24 or 48 hr after a single dose of 200 mg/kg po to male or female rats.

Table 18. Sponsor's "Table 4:" *In vivo* cytogenetics analysis: summary of chromosomal aberration and aberrant cell frequencies.

Compound	Dose (mg/kg)	Time[1] (hrs.)	Total No. Animals Evaluated		Total No. Metaphases Evaluated		Mean Percent Aberrant Cells/ Animal[2] (± Sd)			Mean No. Aberrations/Cell[3]		
			♂	♀	♂	♀	♂	♀	(♂ & ♀)	♂	♀	(♂ & ♀)
Gepirone	200	6	5	5	500	500	.6 ± .5	.6 ± .9	.6 ± .7	.006	.006	.006
		24	5	5	500	500	0	.4 ± .5	.2 ± .4	0	.004	.002
		48	5	5	500	500	.8 ± .8	.2 ± .4	.5 ± .7	.008	.002	.005
0.5% MC[4]	-	6	5	5	500	500	0	.4 ± .5	.2 ± .4	0	.004	.002
		24	5	5	500	500	1.0 ± .7	.4 ± .5	.7 ± .7	.01	.004	.007
		48	5	5	500	500	.6 ± .9	.8 ± .8	.7 ± .8	.006	.008	.007
		Total	15	15	1500	1500	.5 ± .7	.5 ± .6	.5 ± .7	.005	.005	.005
TEM[5]	1.0	24	4	4	200	200	26.0 ± 8.3	34.5±2.2	30.3 ± 6.0	-	-	-

[1] Time from injection of compound to sacrifice.

[2] Percent aberrant cells equals (number of metaphases with at least one structural aberration) divided by (total metaphases evaluated per animal) X 100.

[3] Total structural aberrations, excluding gaps, divided by total number of metaphases evaluated in all animals per dose group. Not including metaphases with greater than 10 structural aberrations. (All metaphases evaluated from animals treated with the test article or negative control had either one or zero structural aberrations.)

[4] MC - methyl cellulose.

[5] Aberrations not completely enumerated due to occasional metaphase with large number of abnormalities.

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D. Additional genotxicity tests

1. ***Study title: Geprione hydrochloride (BMY-13805-1): the hepatocyte primary culture/DNA repair assay using rat hepatocytes.***

Key findings: negative.

Study no: study no. 84-26; accession no. (b) (4)-20277 (identification no. in electronic submission).

Study type: unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes.
Volume #, and page #: volume 42, pages 118-160.
Conducting laboratory and location: (b) (4)

Date of study initiation: 12/10/84 (initial study); 12/17/84 (follow-up study).
GLP compliance: yes, see page 123.
QA reports: yes, see page 123.
Drug, lot #, and % purity: BMY-13805-1; lot no. 15; purity not specified, but assayed at 99.87% (7/23/84).

Formulation/vehicle: dissolved in deionized water to a maximum concentration of 100 mg/ml.

Methods:

Cell line: primary hepatocytes, isolated from adult male F344 rats.
Dose selection criteria:
Basis of dose selection: 12 concentrations across 6 orders of magnitude to a maximum concentration of 1000 μg/ml were used; the highest dose that showed ≤10% cytotoxicity (estimated by trypan blue staining), plus 5 lower doses were analyzed for UDS.
Range finding studies: large range of concentrations in 2 full studies, with concentrations to be analyzed chosen based upon cytotoxicity (estimated from trypan blue-stained cultures and verified by absence of s-phase cells and general morphology in the UDS assay).
Test agent stability: dosing solutions were analyzed and ranged from 89-113% of nominal concentrations in first study and 93-97% in the second study.
Controls:
Vehicle: deionized water (for test article).
Negative controls: pyrene in DMSO, DMSO, and untreated cells.
Positive controls: B (a) P in DMSO.
Comments:
Exposure conditions:
Incubation and sampling times: cells were incubated with drug (test article or control) and ^{3}H-thymidine for 18-20 hr; then rinsed, and processed for autoradiography (NTB emulsion, after swelling nuclei in 1% sodium citrate); exposed for 10 d at 4 degrees C; developed; and stained with H & E.
Doses used in definitive study: 12 concentrations from 0.005 to 1000 μg/ml (see table below).

Study design: See Sponsor's table below.

Table 19. Sponsor's table showing the experimental design used for both UDS assays.

Exposure	Concentrations Tested (In Medium)		Number of Coverslips
BMY-13805-1	1000	ug/ml	3
	500	ug/ml	3
	100	ug/ml	3
	50	ug/ml	3
	10	ug/ml	3
	5	ug/ml	3
	1	ug/ml	3
	0.5	ug/ml	3
	0.1	ug/ml	3
	0.05	ug/ml	3
	0.01	ug/ml	3
	0.005	ug/ml	3
B(a)P	10^{-4}M		3
	$5x10^{-5}$M		3
	10^{-5}M		3
Pyrene	10^{-4}M		3
	$5x10^{-5}$M		3
	10^{-5}M		3
Cell Culture			3
DMSO	1%		3

Analysis:

No. of replicates: triplicate cover slips were analyzed for each treatment group.

Counting method: an Artek Model 880 electronic counter with microscopic attachment was used for grain counting; a minimum of 20 nuclei per slide and 3 slides per dose were scored. Net nuclear counts were determined by subtracting cytoplasmic counts from total nuclear counts (using the highest count from 3 nuclear-sized cytoplasmic areas near each nucleus; to minimize false positives).

Criteria for positive results: The Sponsor stated that the test article was reported as positive "when the minimum net graincount of 5 per nucleus is consistently observed in triplicate coverslips throughout the experiment."

Summary of individual study findings:

Study validity: both assays were valid: negative controls averaged 0 to 0.1; positive control (10 µM B(a)P) was 15.6 ±2.4 (first assay) and 28.1±1.3 (second assay) net grains per nucleus.

Study outcome: There was no indication of increased incorporation of ^{3}H-thymidine into nuclei of cells treated with gepirone in either assay (see table, below).

Table 20. Gepirone did not increase UDS in rat hepatocytes (excerpted from the Sponsor's tables of results).

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1st assay

CONTROLS			TEST RESULTS			
Positive	conc.	Autoradiog. grains/nucleus (NET)	conc. µg/ml	Autoradiog. grains/nucleus (NET)	Cytotoxicity	Evaluation
			1000 "		Toxic	
B(a)P	10^{-5}M	15.6±2.4	500 "		Toxic	
			100 "		Toxic	
			50 "		Toxic	
			10 "	0±0	Non-Toxic	Negative
			5 "	0±0	Non-Toxic	Negative
			1 "	0±0	Non-Toxic	Negative
			0.5 "	0±0	Non-Toxic	Negative
			0.1 "	0±0	Non-Toxic	Negative
Negative	conc.	grains/nucleus (NET)	0.05 "	0±0	Non-Toxic	Negative
			0.01 "		Non-Toxic	
Pyrene	10^{-5}M	0.1±0.1	0.005		Non-Toxic	
Cell Control		0±0				
DMSO	1%	0±0				

2nd assay

CONTROLS			TEST RESULTS			
Positive	conc.	Autoradiog. grains/nucleus (NET)	conc. µg/ml	Autoradiog. grains/nucleus (NET)	Cytotoxicity	Evaluation
B(a)P	10^{-5}M	28.1±1.3	1000 "		Toxic	
			500 "		Toxic	
			100 "		Toxic	
			50 "	0.2±0.1	Slightly toxic	Negative
			10 "	0±0	Non-Toxic	Negative
			5 "	0.1±0.1	Non-Toxic	Negative
			1 "	0.2±0.3	Non-Toxic	Negative
			0.5 "	0.2±0.2	Non-Toxic	Negative
			0.1 "	0±0	Non-Toxic	Negative
Negative	conc.	grains/nucleus (NET)	0.05 "		Non-Toxic	
			0.01 "		Non-Toxic	
Pyrene	10^{-5}M	0±0	0.005"		Non-Toxic	
Cell Control		0±0				
DMSO	1%	0±0				

2. ***Study title: CHO/HGPRT mammalian cell forward gene mutation assay with BMY-13805-1.***

Key findings: Negative under the conditions of this study.

Study no: (b) (4)-314-BR-007-84; accession no. (b) (4)-20150 (identification no. in electronic submission).
Study type: mammalian cell forward gene mutation assay in CHO/HGPRT cells.
Volume #, and page #: volume 42, pages 205-252.
Conducting laboratory and location: (b) (4).
Date of study initiation: 10/31/84; definitive study on 12/6/84.
GLP compliance: yes, see page 229.
QA reports: yes, see page 229.

Drug, lot #, and % purity: BMY-13805-1; lot no. 15; purity not specified, but assayed at 99.87% (7/23/84).

Formulation/vehicle: test article was dissolved in sterile water for injection at maximum concentration of 100 mg/ml.

Methods:

Cell line: CHO-K1-BH4 cells, a Chinese hamster ovary cell line.

Dose selection criteria:

Basis of dose selection: cytotoxicity and preliminary mutagenicity assays (see below).

Range finding studies: Cytotoxicity (i.e., reduction in colony-forming ability) of 5-hr treatment, with 10 concentrations of drug from 0.03 to 1000µg/ml, with and without metabolic activation; 1000µg/ml resulted in 31%, 36%, and 60% survival without S-9, with 2% S-9, and with 10% S-9, respectively. Preliminary mutagenicity assay: 100, 500, and 1000µg/ml, without S-9 and with 2% and 10% S-9: negative.

Test agent stability: dosing solutions from all studies were analyzed; for the definitive study, solutions ranged from 94-101% of their nominal concentrations.

Metabolic activation system: S-9 fraction from livers of rats that had been treated with Aroclor 1254; both 2 and 10% concentrations were tested, 10% in definitive study.

Controls:

Vehicle: sterile water for injection.

Negative controls: untreated and water.

Positive controls: ethylmethanesolfonate (EMS; without metabolic activation), dimethylnitrosamine (DMN; with metabolic activation).

Comments:

Exposure conditions:

Incubation and sampling times: cells were incubated with drug, ± activation, for 5 hr; rinsed and incubated for a further 19 hr, before cytotoxicity or mutagenicity (selection for 6-TG-resistent clones) was determined.

Doses used in definitive study: 100, 300, 600, 800, and 1000µg/ml, with and without 10% S-9.

Analysis:

No. of replicates: duplicate cultures for each condition.

Counting method: after growing for 7 days in the presence of 6-TG, colonies were fixed, stained with crystal violet and counted.

Criteria for positive results: Quoted from the Sponsor below:

"

control. A test substance showing a dose-dependent increase of mutation induction with at least one dose exhibiting a mutation frequency that was greater than or equal to 50 x 10^{-6} per cell was considered a suspect mutagenic response, and the sponsor would be advised to repeat the assay bracketing the positive response dose. A test article showing a true positive response in this assay should also exhibit a dose-response relationship. The spontaneous background mutation frequency (forward mutation frequency) is usually 0 - 10 x 10^{-6}, however, values up to 20 x 10^{-6} are deemed acceptable. Data is "

Summary of individual study findings:

Study validity: valid: negative control (water) did not significantly alter mutation frequency versus untreated control cells. Positive controls gave strong positive signals for increased mutation frequency and also decreased initial survival and decreased cloning efficiency. (See table, below.)

Study outcome: Gepirone, with or without metabolic activation, did not increase mutation frequency in CHO cells under the conditions of this study (see table, below). Gepirone did decrease initial survival, to 56% of untreated controls at 1000 µg/ml, with or without metabolic activation. There was no clear effect of gepirone on cloning efficiency (prior to selection for 6-TG resistant colonies), in contrast to the positive controls.

Table 21. Gepirone, with or without metabolic activation, did not increase mutation frequency in CHO cells. Sponsor's table of results of the definitive study.

Compound	ug/ml	S-9 (±)	Relative Initial Survival (%)[b]	Total No. of Mutants (5 plates)	Cloning Efficiency (%)	Mutant Frequency (10^6 clonable cells)	Mean Mutation Frequency
Untreated	-	-	99.5[a]	7	74.5	9.4	
Untreated	-	-	100.5	5	91.7	5.5	7.5
Untreated	-	+10%	91.4	5	81.2	6.2	
Untreated	-	+10%	110.9	4	87.2	4.6	5.4
H_2O	10	-	97.0	9	77.5	11.6	
H_2O	10	-	89.9	5	64.0	7.8	9.7
H_2O	10	+10%	93.7	6	86.8	6.9	
H_2O	10	+10%	91.2	7	75.5	9.3	8.2
BMY-13805-1	100	-	86.6	6	86.7	6.9	
BMY-13805-1	100	-	84.1	8	69.0	11.6	9.3
BMY-13805-1	300	-	81.9	6	84.2	7.1	
BMY-13805-1	300	-	83.1	4	87.8	4.6	5.8
BMY-13805-1	600	-	68.5	7	79.2	8.8	
BMY-13805-1	600	-	71.5	12	65.8	18.2	13.5
BMY-13805-1	800	-	63.1	5	88.2	5.7	
BMY-13805-1	800	-	85.6	7	97.5	7.2	6.4
BMY-13805-1	1000	-	52.4	6	76.5	7.8	
BMY-13805-1	1000	-	60.4	9	97.8	9.2	8.5
BMY-13805-1	100	+10%	98.7	4	99.7	4.0	
BMY-13805-1	100	+10%	98.6	12	77.8	15.4	9.7
BMY-13805-1	300	+10%	85.6	8	98.0	8.2	
BMY-13805-1	300	+10%	81.0	7	86.3	8.1	8.2
BMY-13805-1	600	+10%	75.2	2	80.3	2.5	
BMY-13805-1	600	+10%	73.3	10	85.8	11.7	7.1
BMY-13805-1	800	+10%	44.6	7	76.3	9.2	
BMY-13805-1	800	+10%	66.6	3	84.8	3.5	6.4
BMY-13805-1	1000	+10%	58.2	6	96.3	6.2	
BMY-13805-1	1000	+10%	53.6	6	81.5	7.4	6.8
EMS	200	-	56.9	175	60.3	290.2	
EMS	200	-	66.3	164	67.0	244.8	267.5
DMN	100	+10%	30.1	208	37.8	550.3	
DMN	100	+10%	23.0	189	34.3	551.0	550.6

[a] Absolute cloning efficiencies ($\bar{x}$) 86.4%.
[b] Relative cell survivals greater than 100% are reported in the text of the report as 100%.

Genetic toxicology conclusions: Although there was no evidence of genotoxicity in any of the tests that were performed, 1 of the 3 tests from standard battery was inadequate. The *in vitro* chromosomal aberration test was negative after 5-hr treatment with gepirone, with or without metabolic activation. However, the study was not valid, because this negative finding should have been followed up with a study using continuous treatment with gepirone (without activation) for ~24 hr (1.5 doubling times), according to current ICH Guidance (1997).

Labeling recommendations:

VI. CARCINOGENICITY:

A. Study title: BMY-13805: A carcinogenicity oral [dietary] bioassay in rats.

Key study findings: Adequate and negative.

Study number: 13805-003-25-87; designated Study (b) (4)-13411 in electronic version of submission.
Volume #, and page #: volumes 1.43 through 1.48, page 179.
Conducting laboratory and location: Bristol-Myers Squibb Company, Mt. Vernon, Indiana.
Date of study initiation: dosing was started on 11-11-87.
GLP compliance: yes, see volume 1.43, page 172.
QA report: yes, see volume 1.43, page 172.
Drug, lot #, and % purity: BMY-13805-1 [gepirone HCl]; original lot 18, batch E84L097, was (b) (4) after week 19 and designated lot 22, batch E88B175.
CAC concurrence: Not applicable; this study was performed in 1987-1989, before CAC concurrence was available.

Study Type: 2-year bioassay.
Species/strain: male and female Crl:CD(SD)BR rats (Charles River Breeding Labs, Portage, MI).
Number/sex/group: 50/sex/dose, 100/sex for controls; 20/sex/dose (not controls) for verification of exposure.
Age at start of study: 5-6 weeks old at start of dosing (approximately 22-30 days old when received from supplier on 10-27-87).
Animal housing: individually housed in wire-mesh cages; 12-hr light/dark cycle; food and water *ad libitum*.
Formulation/vehicle: Purina Rodent Laboratory Chow #5001 (meal).
Drug stability/homogeneity: There was sporadic variability of drug content in the admixtures (outside the limits set at 90-110%), especially at LD, during the initial 19 weeks of dosing that was rectified (b) (4). Stability for 5 weeks as admixture was confirmed, by analyzing samples every 4 weeks.
Methods:

Doses: 0, 4, 16 (increased from 12 after 18 weeks), and 48 (increased from 36 after 18 weeks) mg/kg/d.
Basis of dose selection: 12% decrease in body weight gain in male rats given 24 mg/kg/d in a 6-mo dietary study.
Restriction paradigm for dietary restriction studies: not restricted.
Route of administration: oral, dietary.
Frequency of drug administration: dietary, *ad libitum.*
Dual controls employed: no, but twice as many rats in control groups (100/sex) as in drug-treated groups (50/sex/dose).
Interim sacrifices: none, except when moribund.

Satellite PK or special study group(s): 20/sex/dose (not controls) for verification of exposure.
Deviations from original study protocol: MD and HD increased after 18 weeks of dosing, with intent of achieving meaningful toxicological effects (?).
Statistical methods: Tumor data were analyzed by the Sponsor (Bristol-Myers Squibb PRI) using the methods of Peto and Pike for primary analysis.

Observations and times:

Clinical signs: observed daily; for subtle physical signs and palpable masses at each weighing and every 4 weeks independent of weighing after week 66..
Body weights: weekly, during first 13 weeks, then approximately every 4 weeks thereafter.
Food consumption: weekly, during first 13 weeks, then approximately every 4 weeks thereafter.
Ophthalmoscopic examinations: in each animal, during pretest period and during weeks 52 and 104 of dosing.
Hematology: blood samples during weeks 53, 78, and 104; under non-fasted conditions from 10/sex/dose and 20/sex for controls.
Clinical chemistry: blood samples at terminal necropsy during exanguination; after ~18 hr fast from 10/sex/dose and 20/sex for controls.
Organ weights: adrenals, brain, gonads, heart, kidneys, liver, pituitary, prostate, spleen, thyroid, uterus.
Gross pathology: complete necropsies on all rats; CO_2 anesthesia/exsanguination;
Histopathology: See "Histopathology Inventory " for this NDA (Table 15). Complete except that the following tissues were not specified: cervix, fallopian tubes, gall bladder, harderian gland, larynx, mandibular lymph nodes, nasal cavity, pharynx, rectum, and zymbal gland. Also there were a considerable number of missing tissues for thymus (12-20% in females, 18% in MD males) and male mammary glands (control and LD, 16% each).
Toxicokinetics: 5/sex/dose (not controls), following sacrifice in weeks 5, 27, 53, and 79, approximately 2 hr after removal of feeders (i.e., drug availability); analyzed for parent and a metabolite (1-PP), by HPLC (LLQ = 10 ng/ml) and GC/MS (LLQ = 1.81 ng/ml for gepirone and 1.68 ng/ml for 1-PP) with internal and external standards.

Results:

Mortality: There was no increase in mortality in drug-treated rats; survival to termination (104 weeks) was slightly better in drug-treated rats, especially in females (see table and graph, below). In rats that died prematurely or were sacrificed moribund, the major causes of death were pituitary adenoma and chronic nephritis (see table below).

Table 22. Survival rates for rats treated for 104 weeks with gepirone (from Sponsor's table 6, volume 1.47, pp 310-313).

SEX	DOSE, mg/kg/day			
	controls	4	12→16 (@ wk 19)	24→36 (@ wk 19)
M	56/100 = 56%	34/50 = 68%	32/50 = 64%	34/50 = 68%
F	45/100 = 45%	27/50 = 54%	32/50 = 64%	36/50 = 72%

Figure 8. Dietary gepirone decreased mortality in male and female rats (Sponsor's mortality graphs, volume 1.44, pp 329, 330); controls (1), 4 mg/kg/d (2), 12→16 mg/kg/d (3), 24→36 mg/kg/d (4).

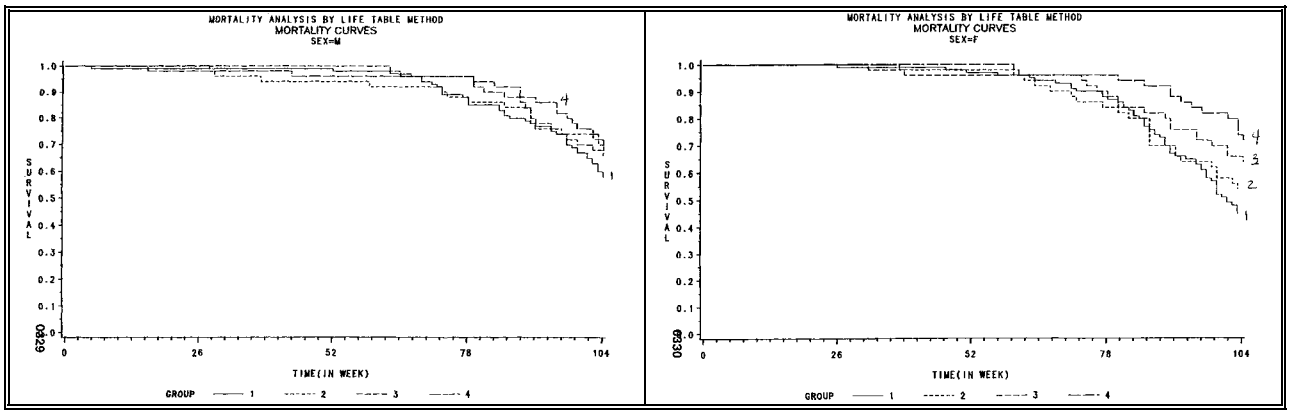


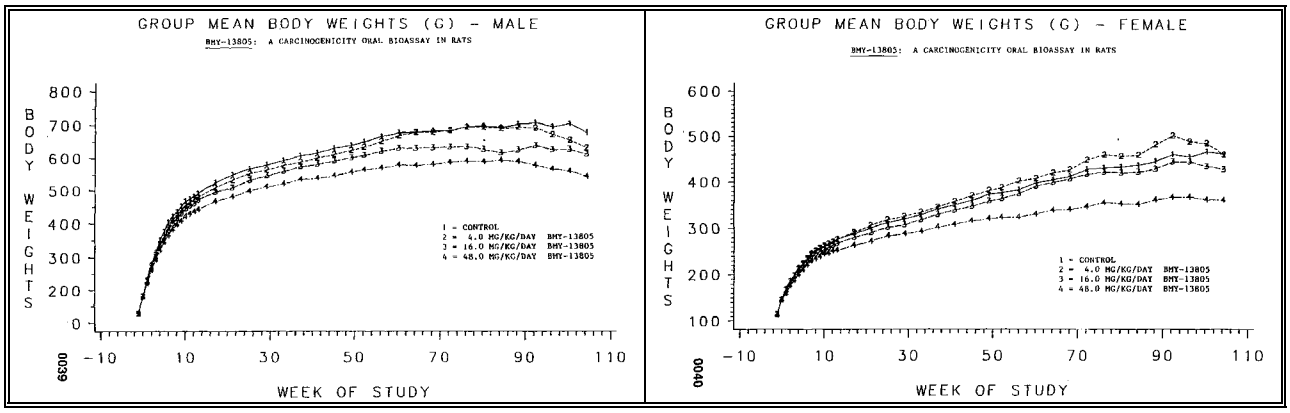
Table 23. Incidences of major causes of deaths or moribund sacrifices (gleaned from the Sponsor's Pathology Table 1, volume 1.43, pp 55-57).

PARAMETER	MALES				FEMALES			
	controls	LDM	MDM	HDM	controls	LDF	MDF	HDF
# of rats	100	50	50	50	100	50	50	50
# dead prematurely	44	16	18	16	55	23	18	14
Pituitary adenomas	11 (25%)	3 (19%)	6 (33%)	3 (19%)	38 (69%)	11 (48%)	9 (50%)	9 (64%)
Chronic nephritis	12 (50%)	3 (19%)	6 (33%)	3 (19%)	2 (4%)	2 (9%)	1 (6%)	1 (7%)
Undetermined	3 (7%)	3 (19%)	0 (0%)	1 (6%)	2 (4%)	0 (0%)	0 (0%)	1 (7%)

Clinical signs: The Sponsor noted a single occurrence of a clonic convulsion in a HDM at week 69, as possibly drug-related. Otherwise, no drug-related clinical signs were noted.

Body weights: [Only group mean data was provided, with statistical analysis (Dunnett's test), not individual animal values.] Mean body weights were decreased in MD and HD males and HD females (see Figure 9, below). For HD males, body weights were decreased 5% after 1 week of dosing, 10% after 9 weeks, 15% after 60 week and 20% after 100 weeks to the end to the study (compared with controls). For MD males, the decreases were less, 4% after 2 weeks, 5% after 5 weeks, 10% after 80 weeks to the end of the study. For HD females, body weights were decreased 6% after 1 week of dosing, 10% after 29 weeks, 15% after 56 week and 20% after 92 weeks to the end to the study (compared with controls).

Figure 9. Dietary gepirone decreased body weights of male and female rats (Sponsor's graphs from volume 1.43, pp 39, 40).



Food consumption: [Only group mean data, as g/kg/day, was provided, with statistical analysis (Dunnett's test), not individual animal values; I calculated average daily consumption, g/d, from mean body weights and g/kg/d food consumption at several (8) weeks during the study.] Males in all treatment groups consumed similar amounts of food throughout the study (ranging from ~19 g/day at the beginning of the study up to ~25 g/day at the end of the study). Females in control, LD and MD groups consumed similar amounts of food throughout the study (ranging from ~15 g/day at the beginning up to ~20 g/day at the end); HD females consumed more across the entire study (~2 g/day more).

Drug intake: Drug intake, calculated at 36 times across the study, appeared to be accurate throughout study: LD group, nominally 4 mg/kg/d, averaged 3.9 g/day (ranged from 3.5 to 4.1 in males and females); MD group, nominally 12 mg/kg/d for 1st 18 weeks, averaged 11.7 for males and 11.8 for females (10.6-13.0), nominally 16 mg/kg from week 19 to termination, averaged 15.9 for males and 15.8 for females (14.6-18.0); HD group, nominally 36 mg/kg/d for 1st 18 weeks, averaged 35.5 for males and 36.4 for females (32.9-37.9), nominally 48 mg/kg/d for week 19 to termination, averaged 47.8 for males and 47.7 for females (41.8-53.8).

Ophthalmoscopic examinations: no treatment-related affects at either time tested.

Hematology: no remarkable effects attributable to drug treatment at times tested (i.e., at weeks 53, 78 and 104, in survivors).

Clinical chemistry: no remarkable effects attributable to drug treatment at week 105 (in survivors).

Organ weights: no notable effects: decreases in absolute weights of several organs in HD groups were attributable to the decrease in body weights at this dose. [NB Two HD males had very high absolute adrenal weights, #18 at 380 mg and #50 at 1212 mg vs average control of ~90 mg. Both these rats showed enlarged (right) adrenals at necropsy; microscopically, #18 had a large, circumscribed, congested pheochromocytoma, #50 had a large, invasive, pleomorphic, anaplastic, malignant pheochromocytoma. But pheochromocytoma was not uncommon in control males (17/100).]

Gross pathology: At necropsy, treatment-related changes were limited to lungs and urinary bladder. The incidence of pale focal areas was increased in the lungs of HD rats: 10% for male and female controls, compared with 30% for HDM and 40% for HDF. The incidence of distention of the urinary bladder of drug treated male rats was increased to 24% at MD and 50% at HD, compared with 9% in controls and 8% in at LD.

Histopathology:

Non-neoplastic: There was an increase in the incidence but not severity (minimal to moderate) of pulmonary histiocytosis at HD; 38% in HDM and 42% in HDF vs 13% in male controls and 19% in female controls.

Neoplastic: The Sponsor found "no biologically significant differences in the total incidence or variety of benign and malignant neoplasms between treated and control rats." However, they did note statistically significant incidence of 2 neoplasms in HD males: interstitial cell adenomas in testes and total hemangiomas (subcutis and liver); see table, below.

In support of the lack of biological significance of the slightly higher incidence of interstitial cell adenomas in HDM, I would add that interstitial cell hyperplasia was noted in 2 controls and 2 MD (slight to moderate severity). Furthermore, the 5 adenomas in the HD group, as well as the 2 in controls and 2 at LD, were all noted at terminal sacrifice; and the 1 adenoma at MD was noted at week 102, when the rat was euthanized due to a large tumor on its head (subsequently determined to be a squamous cell carcinoma and probable cause of moribund condition). Finally, the Charles River website provides incidence tables for this tumor in males of this strain that ranged 1.4-10.0% (1992 report) or 1.43 –7.14 % (2001 report) in free-feeding rats and 1.28-8.47% (1998 report) in food restricted rats.

Regarding the hemangiomas (1 in liver, 1 in subcubitus), livers of all rats were examined, however, the subcubitis of 9 male controls, 3 LDM, 5 MDM and 12 HDH were also examined (presumably because of gross lesions). Additionally, hemangiosarcomas were also observed in mesenteric lymph node and spleen. Combining hemangiomas and hemangiosarcomas in whole body, there is no clear or statistically significant increase in incidence related to drug-treatment (see Table 25, below).

Table 24. Sponsor's table of incidence of statistically significant neoplasms in male rats (from volume 1.43, p 26). P-values were 0.04 for interstitial cell adenomas and 0.02 for hemangiomas.

Text Table 2: Incidence of Statistically Significant Neoplasms in Males

BMY-13805 (mg/kg/day)	0	4	16	48	Literature Historical Ranges (%)*	
Number of Rats	100	50	50	50	Reference 1	Reference 2
Tissue/Neoplasm:						
Testes						
Interstitial Cell Adenoma	2	2	1	5	1.4-10	0-12
Subcutis and Liver (1 each)						
Hemangioma	0	0	0	2	1.4-2.0*	0-1.1*

* Literature historical ranges for Crl:CD®BR Rats:
Reference 1 - Compiled data from 19 groups of control rats
Reference 2 - Compiled data from 11 groups of control rats
* Incidence ranges of hemangioma in subcutis[1] and liver[2].

Table 25. Incidences of hemangiomas and/or hemangiosarcomas in male rats. [Each occurrence is in a unique rat; no rat had these tumors identified in more than 1 tissue/organ.] The Reviewing Statistician determined this apparently increased incidence in HDM to be non-significant, with exact p-value of 0.0911 and asymptotic p-value of 0.0659 (neither adjusted for mortality).

TISSUE	CONTROLS	LDM	MDM	HDM
Liver	0/100	0/50	0/50	1/50
Spleen	1/100	1/50	0/50	1/49
Mesenteric LN	1/100	0/48	0/50	0/49
Subcutis	0/9	0/3	0/5	1/12
Total rats affected	**2/100 (2%)**	**1/50 (2%)**	**0/50 (0%)**	**3/50 (6%)**

Regarding other tumors encountered in this study, the Sponsor says:

"The more common tumors occurring in treated animals at a similar or lower incidence rate than in concurrent control animals included: pituitary adenoma, adrenal cortical adenoma and pheochromocytoma, pancreatic islet cell adenoma, and thyroid C cell adenoma in males and females; pituitary carcinoma, uterine polyp, and mammary fibroadenoma, cystadenoma, and adenocarcinoma in females; and, keratoacanthoma and papilloma of skin and fibroma of the subcutis in males."

Toxicokinetics: I agree with the Sponsor's summary of the TK data: 1) both male and female rats showed detectable levels of gepirone and 1-PP in plasma throughout the study (up to week 79 at least) in all dosage groups; 2) levels of gepirone and 1-PP increased with increasing dose at all times tested; 3) gepirone levels tended to be lower (and 1-PP levels higher) in male compared with female rats. This finding of a sex difference in rats is consistent with the predominant role

of CYP3A4 determined for metabolism in humans. Although there was considerable variability in the plasma levels and a quantitative analysis seems unwarranted, I have included the Sponsor's summary tables for plasma levels below.

Table 26. Plasma levels (ng/ml) of gepirone (top panels) and 1-PP (lower panels) in male and female rats after 5, 27, 53, and 79 weeks of dietary dosing (exerpted/copied from Sponsor's tables 11 and 16 from volume 1.43, pp 287 and 290).

MALE RATS: GEPIRONE

TIME AFTER DOSE ADMIN.	MEAN PLASMA CONCENTRATION								
	4MG/KG-MALE			16MG/KG-MALE			48MG/KG-MALE		
Weeks	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
5	5	0.00	0.0	5	0.49	1.1	5	32.39	16.3
27	5	0.68	0.0	5	5.74	8.3	5	58.85	41.9
53	5	0.38	0.8	4	2.86	1.9	4	166.26	200.4
79	5	0.53	1.2	5	5.63	3.7	4	33.67	12.8

NOTE: VALUES <LLQ = 0

FEMALE RATS: GEPIRONE

TIME AFTER DOSE ADMIN.	MEAN PLASMA CONCENTRATION								
	4MG/KG-FEMALE			16MG/KG-FEMALE			48MG/KG-FEMALE		
Weeks	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
5	5	0.00	0.0	5	1.32	1.9	5	27.38	22.1
27	5	4.52	11.8	5	32.23	24.1	5	65.29	41.0
53	5	1.24	1.9	5	10.19	21.7	4	64.73	12.9
79	5	1.88	5.2	4	35.44	19.2	3	82.59	78.2

NOTE: VALUES <LLQ = 0

MALE RATS: 1-PP

TIME AFTER DOSE ADMIN.	MEAN PLASMA CONCENTRATION								
	4MG/KG-MALE			16MG/KG-MALE			48MG/KG-MALE		
Weeks	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
5	5	17.35	4.3	5	48.54	22.1	5	183.64	64.7
27	5	12.00	5.8	5	94.45	37.3	5	495.18	258.3
53	5	22.26	13.9	4	115.04	53.8	4	504.72	96.7
79	5	15.52	9.9	5	102.81	28.4	4	505.94	344.6

NOTE: VALUES <LLQ = 0

FEMALE RATS: 1-PP

TIME AFTER DOSE ADMIN.	MEAN PLASMA CONCENTRATION								
	4MG/KG-FEMALE			16MG/KG-FEMALE			48MG/KG-FEMALE		
Weeks	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
5	5	4.20	1.3	5	7.98	3.5	5	44.97	21.2
27	5	7.61	4.9	5	38.95	21.4	5	126.98	47.8
53	5	4.76	1.9	5	33.91	18.6	4	147.21	99.4
79	5	8.48	4.0	4	50.83	6.5	3	102.88	41.2

NOTE: VALUES <LLQ = 0

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: This 2-year dietary carcinogenicity study in male and female rats appears adequate. Doses were limited by decreased body weight at the HD (and arguably at the MD in males). From the food consumption data, there is no evidence of a palatability problem; mean food consumption for drug-treated male groups and LD and MD females was the same as for controls and HD females appeared to consume more than controls.

Evaluation of tumor findings: There were no biologically significant tumor findings related to gepirone treatment.

B. Study title: BMY-13805 [gepirone HCl]: Two-year dietary carcinogenicity study in mice.

Key study findings: Adequate and negative.

Study number: (b) (4) Study no. 455-012; B-MS Study no. 13805-30A-25-88; designated Study (b) (4) -51411 in electronic version of submission.
Volume #, and page #: volumes 1.48, page 180 through 1.
Conducting laboratory and location: (b) (4) [Sponsor: Bristol-Myers Squibb Company]. Pathology: Bristol-Myers Squibb Company, Mt. Vernon, Indiana.
Date of study initiation: dosing was started on 8-29-88; terminal necropsy was performed from 8-27 through 8-31-90.
GLP compliance: yes.
QA report: yes.
Drug, lot #, and % purity: BMY 13805-1 [gepirone HCl]; batch no. E88C016 (99.6% pure, used from 7-8-88 to 7-29-90) and batch no. E89L769 (99.0% pure, used from 8-3-90 to 8-24-90).
CAC concurrence: Not applicable; this study was performed in 1988-1990, before CAC concurrence was available.

Study Type: 2-yr bioassay.
Species/strain: Charles River CD-1 (ICR-BR) mice (Charles River Laboratories, Portage, MI).
Number/sex/group: 50/sex/dose, 100/sex for controls; 20/sex/dose (not controls) for verification of exposure.
Age at start of study: 6-7 weeks at start of dosing.
Animal housing: individually housed in wire-mesh cages; 12-hr light/dark cycle; food and water *ad libitum*.
Formulation/vehicle: admixtures of drug ground/blended with Certified Rodent Chow #5002, Purina Mills, Inc; admixtures prepared weekly (stability determined for at least 10 days).
Drug stability/homogeneity: Before initiating the study, admixtures were determined to be stable for at least 10 days. During the study, admixtures for all doses (3 sampels per dose/concentration admixture) were assayed weekly in weeks 1-4, every 4 weeks thereafter and appeared to be accurate (averaging 98-106% of nominal concentrations across all dose groups). Homogeneity was determined for low and high dose admixtures (10 x 50 g samples per admixture concentration) at weeks 24, 52 and 76 (and at week 80 for the HD male admixture, after the increase in dose/concentration at week 79) and the admixture preparation appeared reliable (coefficients of variation averaged 8.8%, 3.2% and 0.9% for the 5 mg/kg/d, 250 mg/kg/d, and 350 mg/kg/d doses).
Methods:

Doses: For males: initial doses were 0, 5, 15, and 50 mg/kg/d; 15→25 at week 7; 50→100→150→250→350 at weeks 7, 12, 19, and 79, respectively. For females: initial doses were 0, 5, 25, and 75 mg/kg/d; 75→100→150→250 at weeks 7, 12, and 19, respectively.

Basis of dose selection: Initial dose selection was based upon the results of a 13-week dietary range-finding study [study (b) (4)-13082, aka 13805-30A-11-87, aka (b) (4)-74004; conducted by (b) (4).; male and female Crl:CD-1(ICR)BR mice, 19/sex/dose, dietary, drug batch no. 18]. The low dose of 25 mg/kg/d produced no observable toxicity. Doses of 50, 100 and 200 mg/kg/d produced significant decreases in body weight gains in male rats, 12%, 20% and 25% lower than controls, respectively. Doses of 100 and 200 mg/kg/d produced decreases in body weight gains of female rats, 12% and 14% lower than controls, respectively (see table below). Based upon these decreases in body weight gains after 13 weeks of dosing, MTDs for 2-year carcinogenicity study would be predicted to be 50 mg/kg/d for male and 100 mg/kg/d for females.

Table 27. Gepirone decreased body weights and body weight gains in male and female mice in a 13-week range finding study. Values represent mean weights for groups, in grams; transposed from Sponsor's table.

SEX	WEEK (PARAMETER)	DIETARY DOSE, MG/GK/D				
		0	25	50	100	200
M	1	26.9	27.1	27.0	27.1	27.1
	13	37.1	35.4	34.1	32.1*	30.7*
	(BW vs control)	100%	95%	92%	87%	83%
	(BWG)	+38%	+31%	+26%	+18%	+13%
F	1	21.9	22.0	22.0	22.0	22.0
	13	28.1	28.6	27.8	25.6	25.1*
	(BW vs control)	100%	102%	99%	91%	89%
	(BWG)	+28%	+30%	+26%	+16%	+14%

Restriction paradigm for dietary restriction studies: not restricted.
Route of administration: oral, dietary.
Frequency of drug administration: dietary, *ad libitum.*
Dual controls employed: no, but twice as many mice in control groups (100/sex) as in drug-treated groups (50/sex/dose).
Interim sacrifices: none, except where moribund.
Satellite PK or special study group(s): 24/sex/group (not controls) for verification or exposure.
Deviations from original study protocol: some of the initial doses were increased.
Statistical methods: Mortality data and combined (fatal and incidental) tumor data were analyzed by Peto and Pike trend test (with $p<0.05$ accepted as significant). Analysis was blocked by sex and tumor data was based upon the number of mice with tumors, not the number of tumors.

Observations and times:

Clinical signs: mortality, moribundity, and overt toxicity at least twice daily; detailed observation at least once per month for 1st 14 mos, then twice monthly thereafter.
Body weights: twice pre-test, weekly for weeks 1-14, every 2 weeks for weeks 15-30, then monthly.
Food consumption: ???
Ophthalmology exams: all main study mice pre-test, and at 12 and 24 mos.

Hematology: all surviving mice immediately prior to terminal sacrifice at necropsy.
Clinical chemistry: ???
Organ weights: at terminal sacrifice on all surviving mice from main study; liver (with gallbladder), kidneys, heart, spleen, ovaries, testes, and brain (with stem).
Gross pathology: all mice from main study (surviving mice at terminal necropsy in week 105, as well as premature decedents).
Histopathology: See "Histopathology Inventory " for this NDA (Table 15). Complete except that the following tissues were not specified in the reports: fallopian tubes, harderian gland, lachrymal gland, larynx, mandibular lymph nodes, nasal cavity, pharynx, and zymbal gland. Also there were a considerable number of missing tissues for thymus (30-50%), male mammary glands (45-60%), thyroids (16% for HDM and control females), cervical LN (10-30% for males and 8-14% for females), and female mesenteric LN (8-12 % for controls and LDF and MDF).
Toxicokinetics (verification of exposure): blood samples from 6 mice per group (where possible) at 1, 6, 12, and 18 mos.

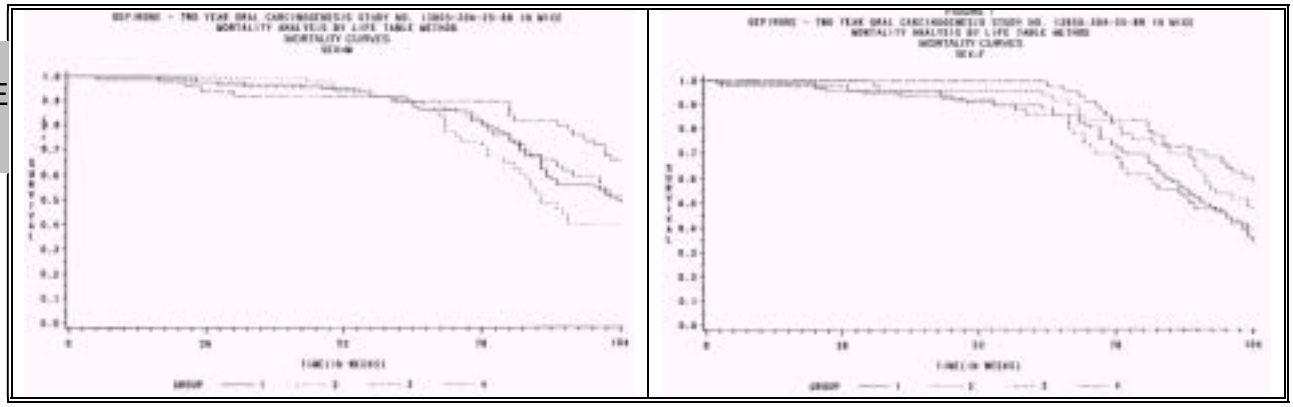
Results:

Mortality: No drug-related adverse effects on mortality were observed. Drug-treatment seemed to improve survival, especially at the HD (see table and figure below).

Table 28. Survival rates for mice treated for 104 weeks with gepirone (transposed from Sponsor's table).

SEX	DOSE			
	controls	LD	MD	HD
M	49/100 = 49%	20/49 = 40%	26/50 = 52%	33/50 = 66%
F	34/100 = 34%	24/50 = 48%	20/50 = 40%	28/50 = 56%

Figure 10. Dietary gepirone tended to decrease mortality in HD male and female mice (Sponsor's mortality graphs).



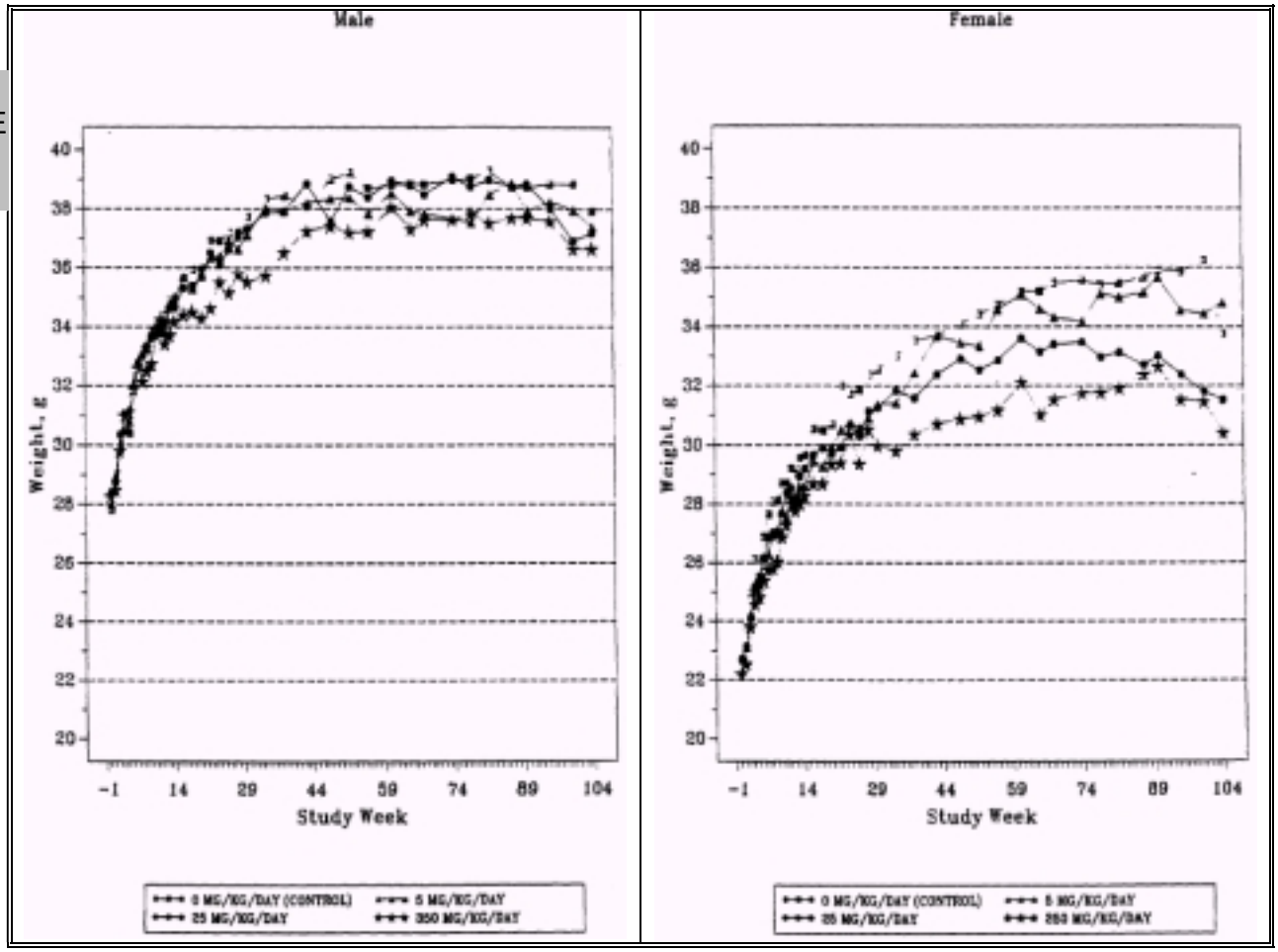
Clinical signs: No drug-related findings.

Body weights: Decreased body weights were observed in females treated at MD (evident by week 26 (9% decrease vs controls) and 10% lower than controls at week 100) and HD (evident by week 13 (9% decrease vs controls) and 17% lower than controls at week 100) (see table and figure, below).

Table 29. Gepirone slightly decreased body weights in HD female mice (transposed from Sponsor's table). Body weights are presented as group means and are expressed in grams.

SEX	WEEK	DOSE GROUP			
		controls	LD	MD	HD
M	1	29	29	29	29
	13	35	35	35	34
	26	37	37	37	35
	100	39	38	37	37
F	1	24	23	23	23
	13	30	29	29	28*
	26	32	31*	30*	29*
	100	36	34	32*	30*

Figure 11. Gepirone slightly decreased body weights in HD female mice (Sponsor's figures).

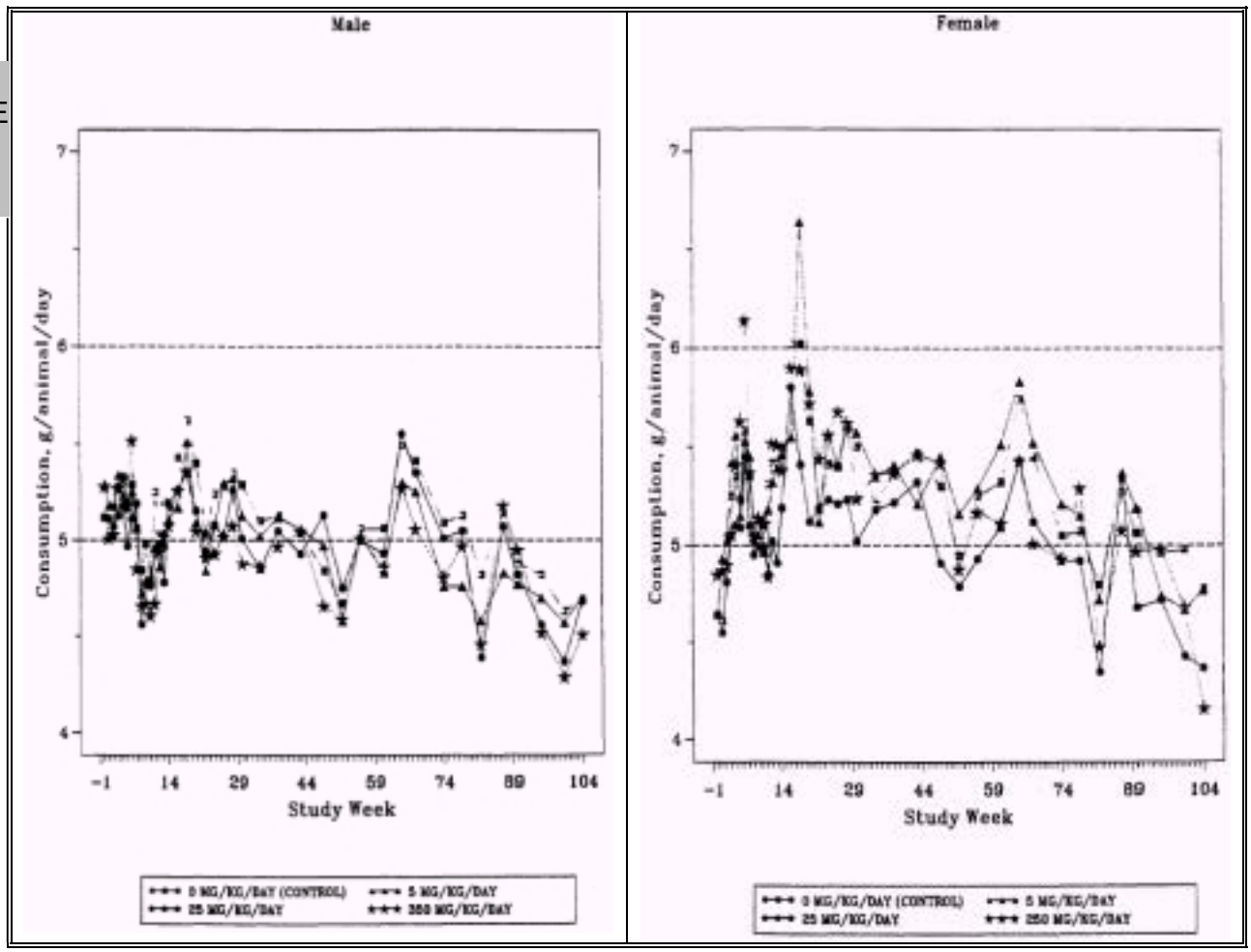


Food consumption: There was no consistent or dramatic effect of gepirone treatment on food consumption in male or female mice (see table and figure, below).

Table 30. Gepirone did not appreciably or consistently alter food consumption in mice (transposed from Sponsor's table). Food consumption is presented as group means and is expressed in grams/mouse/day.

SEX	WEEK	DOSE GROUP			
		controls	LD	MD	HD
M	1	5.0	5.2	5.1	5.0
	13	5.2	5.0	4.8	5.0
	26	5.3	5.3	5.0	5.0
	100	4.6	4.6	4.4	4.3
F	1	4.6	4.9	4.6	4.9
	13	5.5	5.4	4.9	5.5
	26	5.4	5.4	5.2	5.6
	100	5.0	4.7	4.4	4.7

Figure 12. Gepirone did not appreciably or consistently alter food consumption in mice (Sponsor's figures).



Hematology: only analyzed at terminal sacrifice; only finding was a slight (9%) decrease in hemoglobin and hematocrit in MDM.

Clinical chemistry: not performed.

Organ weights: no changes in males; significantly decreased absolute heart weights in MDF and HDF (↓17% vs controls) and absolute kidney weights in MDF (↓13% vs controls), but organ weights were not different from controls when corrected for decreased body weights (body weights ↓9% vs controls).

Gross pathology: limited to apparently increased incidence of corneal opacities (22% in controls, 50% at LD, 48% at MD, and 43% at HD) and thickening of glandular stomach (6% in controls, 0% at LD, 16% at MD, and 19% at HD) in dosed males surviving to termination. The incidence of corneal opacities was approximately 50% in all female groups. There was a hint of increased incidence of thickening of the glandular stomach in HDF (14% vs 6% in controls, 8% at LD and 0% at MD).

Histopathology:

Non-neoplastic: 1) dose-related increase in incidence of inflammation of fundic stomach in males (0/100 in conrtrols, 4% at LD, 4% at MD, and 10% at HD) and females (1/98 in controls, 0% at LD, 2% at MD and 8% at HD). 2) dose-related decreased incidence (but not severity, minimal to moderate) of progressive murine nephropathy in males (68% in controls, 55% at LD, 50% at MD, and 44% at HD).

Neoplastic: The Sponsor found no statistically significant increases in incidences of any tumors attributable to gepirone dosing. However, 2 tumors were nearly significant ($p<0.08$): adrenal pheochromocytomas in females (2/~50 HDF vs 0/100 controls, see table below) and hemangiosarcomas (alone or combined with hemangiomas) in males (see table below). The (non-significant) 4% incidence of pheochromcytomas in HDF seems likely to be spurious; Charles River (1995 report) gives a spontaneous incidence in females of this strain ranging from 0.5-2%. The slight (non-significant) increase in hemangiomas and/or hemangiosarcomas in dosed males would also seem to be spurious; Charles River did not give an incidence of these tumors in mouse whole body, but the incidences for hemangiosarcomas (the more tumor with higher incidence) in liver and skin ranged from 0-2.8 and 0-5.6, respectively, in this strain of male mice.

Table 31. Sponsor's Peto and Pike analysis of adrenal tumors in female mice.

Pheochromocytomas (Adrenal only)					
Group	1	2	3	4	p-value
Fatal	0	0	0	0	
Incidental	0	1	0	2	
Total	0	1	0	2	0.08

Table 32. Sponsor's Peto and Pike analysis of hemangiosarcomas (alone and combined with hemangiomas) in male mice.

Hemangiosarcomas					
Group	1	2	3	4	p-value
Fatal	0	3	3	1	
Incidental	2	0	2	3	
Total	2	3	5	4	0.07
Hemangiosarcomas and hemangiomas combined					
Group	1	2	3	4	p-value
Fatal	0	3	3	1	
Incidental	3	0	4	3	
Total	3	3	7	4	0.08

Table 33. Incidences of hemangiomas and/or hemangiosarcomas in male mice. [Some mice had these tumors identified in more than 1 tissue/organ.]

TISSUE	CONTROLS	LDM	MDM	HDM
Liver	2/100	3/50	6/50	2/50
Spleen	2/100	0/49	2/50	1/50
Testes	0/100	0/49	1/49	0/50
Tail	0/3	0/4	0	1/2
Ear	1/100	0	0	0
Total mice affected	**3/100 (3%)**	**3/49 (6%)**	**7/50 (14%)**	**4/50 (8%)**

Toxicokinetics/verification of exposure: detectable levels of gepirone or its major metabolite 1-PP (see table below) indicated that mice were exposed to gepirone during the study.

Table 34. Sponsor's tables showing plasma concentrations of gepirone and 1-PP in male and female mice treated with geprione in their diet in the 2-year carcinogenicity study.

GEPIRONE STUDY GEP #455-012 (SFA232)
PLASMA CONCENTRATION OF GEPIRONE (NG/ML)
2 YEAR CARCINOGENICITY STUDY IN MICE

TIME AFTER DOSE ADMINISTRATION MONTHS	PLASMA CONCENTRATION - MALE LOW DOSE	MED DOSE	HIGH DOSE
1	LLQ	LLQ	LLQ
6	LLQ	LLQ	LLQ
12	LLQ	LLQ	4.76
18	LLQ	2.25	11.87

GEPIRONE STUDY GEP #455-012 (SFA232)
PLASMA CONCENTRATION OF GEPIRONE (NG/ML)
2 YEAR CARCINOGENICITY STUDY IN MICE

TIME AFTER DOSE ADMINISTRATION MONTHS	PLASMA CONCENTRATION - FEMALE LOW DOSE	MED DOSE	HIGH DOSE
1	LLQ	LLQ	LLQ
6	LLQ	LLQ	6.52
12	LLQ	LLQ	29.23
18	LLQ	LLQ	6.60

GEPIRONE STUDY GEP #455-012 (SFA232)
PLASMA CONCENTRATION OF 1-PP (NG/ML)
2 YEAR CARCINOGENICITY STUDY IN MICE

TIME AFTER DOSE ADMINISTRATION MONTHS	PLASMA CONCENTRATION - MALE LOW DOSE	MED DOSE	HIGH DOSE
1	2.37	4.01	15.85
6	1.88	21.71	482.90
12	3.74	27.57	166.15
18	2.29	24.94	383.40

GEPIRONE STUDY GEP #455-012 (SFA232)
PLASMA CONCENTRATION OF 1-PP (NG/ML)
2 YEAR CARCINOGENICITY STUDY IN MICE

TIME AFTER DOSE ADMINISTRATION MONTHS	PLASMA CONCENTRATION - FEMALE LOW DOSE	MED DOSE	HIGH DOSE
1	LLQ	3.16	35.86
6	2.28	11.68	121.55
12	2.61	12.98	171.45
18	3.05	17.32	292.03

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: This 2-year dietary carcinogenicity study in male and female mice appears to be adequate. Doses were limited by decreased body weight at the HD in females. Although there seemed to be no dose-limiting toxicity in males in this study, the decreases in body weight gains after 13 weeks of dosing in a range-finding study would have predicted an MTD for males of 50 mg/kg/d for the 2-year carcinogenicity study. I presume that the gradual escalation of the high dose from 50→100→150→250→350 at weeks 7, 12, 19, and 79, respectively, allowed for tolerance to develop to the expected decrease in body weight. There was a hint of a similar effect for females; the results of the 13-week range-finding study would have predicted an MTD of 100 mg/kg/d for females for the 2-year carcinogenicity study. However, the high dose was escalated from 75→100→150→250 at weeks 7, 12, and 19, respectively, in the 2-year study, and body weights for the HD females were only 13% lower than controls at week 100. From the food consumption data, there is no evidence of a palatability problem.

Evaluation of tumor findings: There were no tumor findings related to gepirone treatment.

Carcinogenicity conclusions:

Gepirone was adequately tested in rats and mice in 2-year dietary cacinogenicity studies and found not to be carcinogenic at daily doses of up to 48 mg/kg/d in rats and up to 250 mg/kg/d in female mice and 350 mg/kg/d in male mice. These studies were presented to the Executive-CAC on January 8, 2002; meeting minutes are appended in Appendix section IX.

Labeling Recommendations:

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

A. (Segment I) studies of fertility and early embryonic development to implantation in rats.

1. ***Study title: Segment I reproductive study in the rat with BMY 13805-1.***

Key study findings: Although identified by the Sponsor as a Segment I study, this is actually a combined study with dosing of F0 males (prior to and through mating) and females (prior to and through mating, pregnancy, and lactation) and investigation of fertility of F0 rats and development of F1 offspring through mating, pregnancy and weaning of F2 pups.

Fertility and early embryonic development (Segment I):
- Increased latency to mating at 150 mg/kg
- No effects on number of corpora lutea, implantation sites, live or dead fetuses
- Decreased fetus weights at 27 and 150 mg/kg
- Decreased fetus lengths at 150 mg/kg

Prenatal and postnatal development including maternal (F0) function (Segment III-like):
- No effects on length of gestation (F0)
- Increased stillbirths and decreased live litter size at 150 mg/kg
- Increased early postnatal deaths at 150 mg/kg
- Decreased pup birth length at 150 mg/kg
- Decreased pup birth weights at 27 and 150 mg/kg
- Decreased pup weights through weaning and to 14 weeks of age at 150 mg/kg
- Decreased food consumption after weaning at 150 mg/kg
- Increased latency to develop righting reflexes at 150 mg/kg
- Decrement in learning and memory retention trial (but no effect on acquisition) at 150 mg/kg

Other parental effects:
- Decreased body weights in males at 150 mg/kg
- Decreased maternal weights at post-natal days 15-22, with decreased food consumption, especially at post-natal days 1-22, at 150 mg/kg
- Effects on ovary and uterus weights of F0 females (without histopathology) that might indicate endocrine effects at 150 mg/kg.

Study no.: 0430251; accession no. (b) (4)-11485.
Volume #, and page #: volume 57, pages 3-352, and volume 58, pages 2-319.
Conducting laboratory and location: Bristol-Myers Co, Aichi, Japan.
Date of study initiation: not specified, however, rats were received on 4/22/85 (males) and 6/10/85 (females) and were used after 1 week of acclimation.
GLP compliance: yes, see volume 57, pages 65, 66.
QA reports: yes, see volume 57, pages 65, 66.
Drug, lot #, radiolabel, and % purity: BMY 13805-1, lot no. 18, purity not specified.

Formulation/vehicle: dissolved in distilled water.

Methods:

Species/strain: Male (5-week old) and female (9-week old) Sprague-Dawley rats (Crj:CD, Charles River, Japan).
Doses employed: 0, 5, 27, and 150 mg/kg/d for 63 days prior to mating and during mating for males, for 14 days prior to mating, during mating, and throughout gestation and lactation for females.
Route of administration: oral, gavage (10 ml/kg/d).
Housing: Rats were individually housed (except during mating) in suspended metal cages, with food and water *ad lib*.
Study design: Males were dosed for 63 days prior to mating (and throughout mating), females for 14 days prior to mating (and throughout mating, gestation, and lactation); for mating, they were paired (1 male/1 female) for up to 14 days; sperm in vaginal smears indicated day 0 of gestation. Females that had not mated in the original 14-day paring were randomly assigned to one of the males that had mated (this second pairing was for 7 days); males that hadn't mated were re-paired with untreated females. Half the female rats (i.e., even-numbered F0 females, 12/dose) were sacrificed on presumed gestational day 20 (Segments I and II). The other female rats (i.e., odd-numbered F0 females, 12/dose) were allowed to litter normally and to nurture their offspring (F1 offspring, Segment III). F1 offspring were followed/tested through reproductive evaluation when they were 12 weeks old (pairing 1 male/1 female per litter, no brother/sister pairings).
Number/sex/group: 24/sex/group, but only 12/group were used for Segment I/II.
Parameters and endpoints evaluated: *F0 males and females:* body weights, food and water consumption, reproductive performance, necropsy (males at end of dosing, i.e., after mating; females at gestational day 20 or post-natal day 22) and organs weighed*; F1 offspring:* physical landmarks: pinnae detachment (from PN2), presence of hair (from PN3), incisor eruption (from PN8), eye opening (from PN11), testicular descent (from PN20), vaginal opening (from PN29); physical and behavioral tests during pre-weaning (all tests on all pups): surface righting (from PN4-12), air righting (from PN12-17), auditory startle (from PN10-15), visual placing (from PN15-22), olfactory orientation (from PN9-15), negative geotaxis (from PN6-12); physical and behavioral tests during post-weaning (each test on 1/sex/each of 5 litters/group): water T-maze [on PN25, 26 (acquisition), 35 (retention); 5 trials/day]; motor activity [overnight (16 h), individually caged (novel environment), once between PN50-58]; and mating (at ~12 weeks old).

Results:

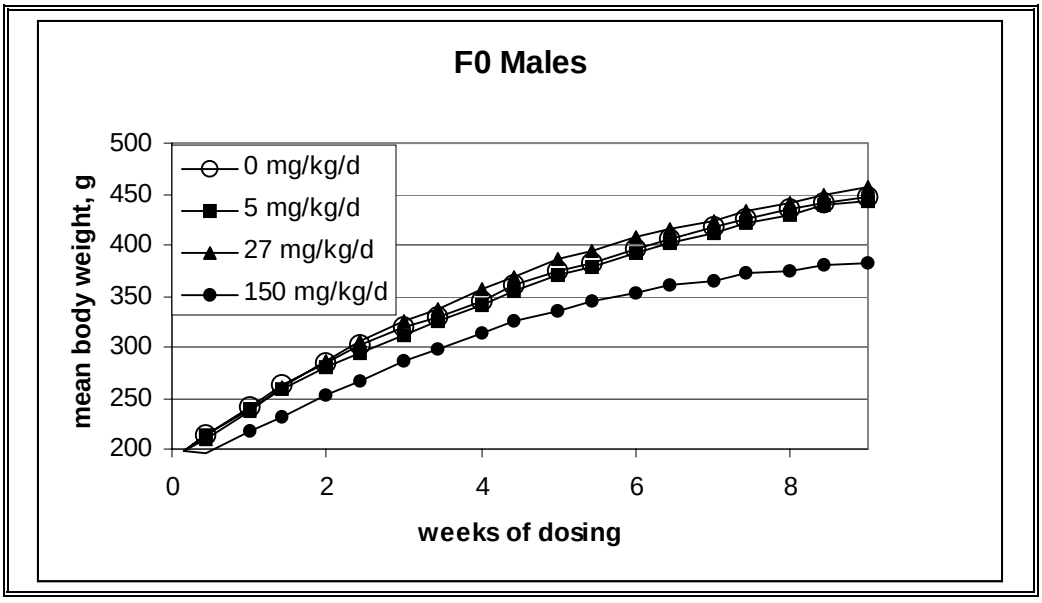
In-life observations in dams and sires (F0):

Mortality of F0 rats: Only two rats (both females, 1 control, 1 HD) died, accidentally, during the study [one each on days 5 (HD #401, 10 min after dosing) and 12 (control #117, 5 min after dosing) of gestation].

Clinical signs in F0 rats: Decreased activity was observed in all (24/24) males and females at HD immediately after dosing on days 1-11. Convulsions were observed in 1 HDF for ~10 min starting ~15 min after dosing on day 2.

Body weight of F0 rats: *Pre-mating:* Mean body weights of HD male F0 rats were 8-15% (significantly) lower than controls throughout the 9-week pre-mating treatment period (see Figure 13, below). Mean body weights of female rats were unaffected by the 2-week pre-mating dosing. *Gestation*: no remarkable effects (HD dams weighed ~6% less than controls at GD20). *Post-partum*: no differences in F1 dam weights through day 8 post-partum, but weights of HD dams were (apparently non-significantly) decreased 8-11% compared with controls from day 15 through weaning at day 22. This decrease reflects the fact that control dams gained weight during this time, but drug-treated dams did not; during this time, drug-treated dams also consumed less food than control dams (see below).

Figure 13. Gepirone (150 mg/kg/d, oral gavage) decreases body weights in F0 male rats during 9-week dosing before mating. [Graphed from mean values in Sponsor's summary table.]



Food consumption of F0 rats: HD male rats ate (3-6 g/day or ~10-20%) less than controls throughout pre-mating dosing period. HD dams ate slightly (2-3 g/day or ~15%) less than controls on gestational days 1-10. HD dams also ate considerably (5-19 g/day or ~20-40%) less than controls on post-partum days 1-22.

Toxicokinetics: not performed.

Mating and Fertility of F0 rats: Nearly all females mated and nearly all matings produced pregnancies, regardless of treatment (see Table 35, below). Although nearly all rats mated during the 2-week pairing period, HD rats took longer to mate than controls (see Figure 14, below). There was a suggestion of increased pre-implantation loss with drug-treatment, but this appeared

due to increased numbers of corpora lutea, rather than decreased number of implantations. There was a hint of a decrease in pregnancies at HD (87% of matings vs 96% in controls; 83% of pairings, vs 96% of controls) that did not reach statistical significance (Chi-square test for trend, p-value = 0.0986). The average litter size at day 20 of gestation was unaffected by treatment. However, MD and HD dams had smaller fetuses: fetuses from HD dams were 10% shorter and weighed 24% less than controls; fetuses from MD dams weighed 10% less than controls (see Table 36, below).

Table 35. Reproductive performance for all F0 dams (sacrificed at GD 20 or delivered at term).

PARAMETER	DOSE, mg/kg/d po			
	0	5	27	150
# of rats paired, per sex	24	24	24	24
# of matings	24	24	24	23
Matings/pairings	100%	100%	100%	96%
# of pregnancies	23	23	22	20
Pregnancies/matings	96%	96%	92%	87%
Pregnancies/pairing	96%	96%	92%	83%

Figure 14. Geprione treatment decreases rate of mating in rats. Values represent the cumulative number of (treated) females that were inseminated (as determined by the Sponsor) during up to 14 days of pairing with (treated) males (not tabulated by Sponsor, see mating records, volume 57, pages 151-154).

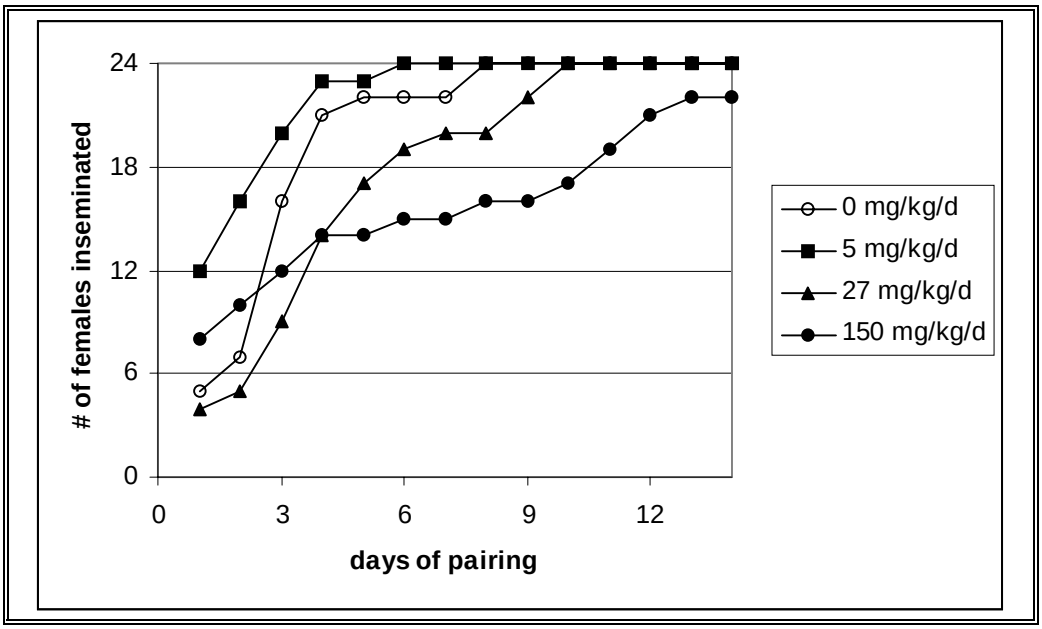


Table 36. Pre-natal development of F1 fetuses (F0 dams sacrificed at gestational day 20).

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Groups	Control	BMY 13805-1 (mg/kg/day, p.o.) 5	27	150
No. of dams examined	11	11	11	12
Corpora lutea				
Total	165	180	208	204
Mean ± S.D. per litter	15.0 ± 1.0	16.4 ± 1.3	18.9 ± 3.9 **	17.0 ± 2.4
Implantations				
Total	159	168	175	175
Mean ± S.D. per litter	14.5 ± 1.1	15.3 ± 1.4	15.9 ± 2.0	14.6 ± 2.8
Dead fetuses				
Empty implantation sites	6	17 *	8	20 *
Early resorption sites	0	0	3	1
Late resorption sites	0	8 **	1	0
Total	6	25 **	12	21 *
Live fetuses				
Total	153	143	163	154
Mean ± S.D. per litter	13.9 ± 1.5	13.0 ± 3.5	14.8 ± 2.6	12.8 ± 3.0
Sex ratio (males/females)	1.10(80/73)	1.34(82/61)	0.99(81/82)	1.41(90/64)
Placental weight ± S.D., g	0.51 ± 0.04	0.55 ± 0.03	0.52 ± 0.04	0.60 ± 0.03
Fetal weight ± S.D., g	3.95 ± 0.24	3.92 ± 0.28	3.64 ± 0.24 *	3.80 ± 0.33 **
Crown rump distance ± S.D., cm	3.78 ± 0.11	3.75 ± 0.06	3.68 ± 0.12	3.80 ± 0.13 **
Tail length ± S.D., cm	1.51 ± 0.03	1.50 ± 0.04	1.49 ± 0.03	1.61 ± 0.05 **
Gross anomalies	0	0	0	0

* p<0.05, ** p<0.01 : Significant difference from controls (Multiple comparison method of Dunnett).
# p<0.05, ## p<0.01 : Significant difference from controls (Chi square test or Fisher exact test).

Reproductive performance of dams that went to term: In dams that went to term, there was a dramatic increase in stillbirths for HD dams (11% of implantation sites vs 0.6 % in controls) and concomitant decrease in live births (78% of implantation sites vs 97% for controls) (see Table 36, below). Furthermore, early post-natal loss was dramatically increased in pups from HD dams (see Table 37 and section on F1 mortality, below).

Table 37. Reproductive evaluation of F0 dams that went to term.

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Groups	Control	BMY 13805-1 (mg/kg/day, p.o.) 5	27	150
No. of dams	11	12	11	7
Length of gestation Mean ± S.D., day	21.5 ± 0.5	21.6 ± 0.5	21.7 ± 0.5	22.0 ± 0.6
No. of implantation sites Total	164	170	174	100
Mean ± S.D.	14.9 ± 2.0	14.2 ± 1.3	15.8 ± 2.0	14.3 ± 2.3
No. of stillborns (% per implantation sites)	1 (0.6)	0	2 (1.1)	11 (11.0) **
No. of alive pups on PN1 Mean ± S.D.	14.4 ± 2.0	13.5 ± 1.6	14.2 ± 2.9	11.1 ± 2.6 *
Sex ratio (males/females)	0.88 (14/84)	0.78 (71/91)	1.05 (80/76)	0.86 (36/42)
Littering index	91.7	100	91.7	58.3
Implantation index	96.3	95.3	89.7 *	78.0 **
Survival index	100	96.9	98.9	38.9 **
Lactation index	100	97.9	98.9	91.3 *
Fertility index	119.7	122.7	118.2	59.1

* p<0.05 : Significant difference from controls (Multiple comparison method of Dunnett).
# p<0.05, ## p<0.01 : Significant difference from controls (Chi square test or Fisher exact test).
Fertility index was not analyzed statistically.
Abbreviation:
Littering index - (No. live litters/No. mated) x 100
Implantation index - (Total births/Implantation sites) x 100
Survival index - (No. pups alive on Day 22/No. pups born alive) x 100
Lactation index - (No. pups alive on Day 22/No. pups alive on Day 4) x 100
Fertility index - (Total pups/No. mated) x 100/11

Table 38. More detailed examination of early post-natal death of F1 pups from parents treated with gepirone. [Calculated from raw data on individual litters.]

PARAMETER	DOSE, mg/kg/d po			
	0	5	27	150
Litters/matings	**92%**	**100%**	**92%**	**58%***
# of dams examined	11	12	11	7
Live pups on day 1/litter	**14.4**	**13.5**	**14.2**	**11.1***
Live pups on day 4/litter	14.4	ND	ND	3.9
Live pups on day 22/litter	14.4	ND	ND	3.7
% alive on day 4/day 1	100%	ND	ND	44%
% alive on day 22/ day 4	100%	98%	99%	91%
% alive on day 22/day 1	100%	97%	99%	39%**
Mean live pup weights, g, PN day 1, M,F	5.95, 5.65	6.07, 5.73	5.80, 5.51	5.13**, 4.88**

*, **: p<0.05, p<0.01, versus controls, by Dunnett's test, Chi-square test, or Fisher's exact test.
ND: not determined; from Sponsor's calculations of survivals at between other days, it is clear that there was no early postnatal mortality in these groups.

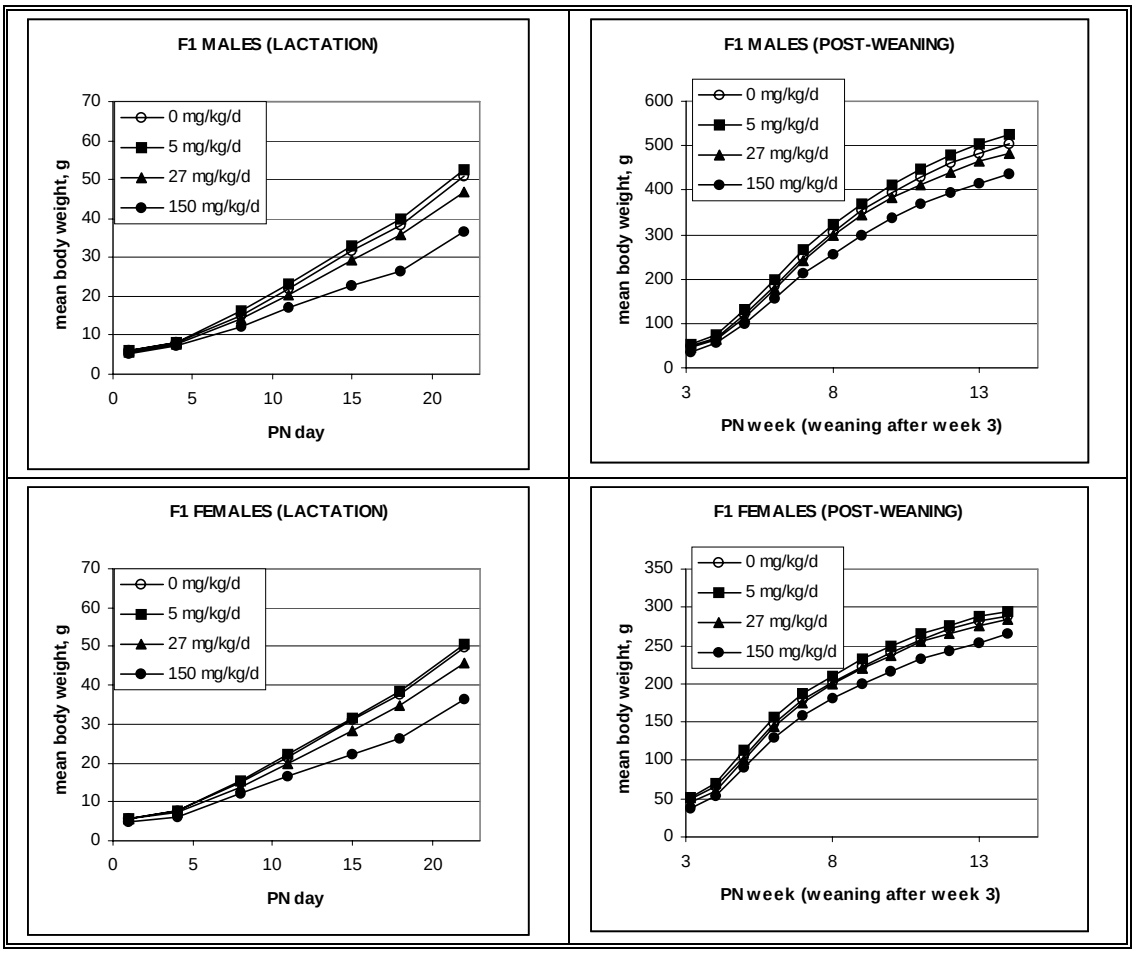
Necropsy of F0 rats: Weights of several organs were altered in HD rats compared with controls, without abnormal findings on gross examination (histopathology was not examined); body weights were lower at HD, especially for males. Adrenal weights were higher in HD males (absolute ↑11%, relative ↑27%) and in HD dams (after ~8 weeks of drug dosing) at weaning (absolute ↑22%, relative ↑32%), but not 3 weeks earlier at term. HD dams had higher ovary weights (↑~12%, absolute and relative) at term, but lower ovary weights (absolute ↓21%, relative ↓13%) and higher uterus weights (absolute ↑38%, relative ↑58%) at weaning. Thymus weights were lower (25-40%) in HD dams at term and at weaning. Liver weights were slightly lower in HD males only (absolute ↓21%, relative ↓10%).

In-life observations, F1 offspring:

Post-natal mortality of F1 offspring: Early post-natal mortality was high in litters from HD dams (see Table 37, above). Essentially all pups that were born alive from control, LD and MD dams survived through weaning. In contrast, more than 50% of pups from HD dams died during the first 4 days after birth. The Sponsor suggests that this may be due to neglect by HD dams, without real evidence offered.

Body weights and food consumption of F1 offspring: Body weights of F1 pups from HD gepirone-treated dams and sires (F0) had lower body weights at birth (~15% lower than controls), during lactation (10-30% lower than controls), and after weaning until at least 14 weeks of age (HD males 13-18% lower than controls and HD females 8-17% lower than controls throughout this period) (see Figure 15, below). These decreases in body weight reflected decreased food consumption; an average of 2-4 g less for males and ~2 g less for females throughout the first 6 weeks after weaning.

Figure 15. Geprione (150 mg/kg/d) treatment of F0 generation (sires and dams) results in decreased body weights of male and female F1 pups, at birth, during lactation, and to at least 14 weeks of age.



Physical landmarks and behavioral reflexes in F1 pups: There were no remarkable changes in these parameters attributable to drug treatment (see Table 39, below). However, there was a suggestion of dose-related increase in latency to develop surface righting and air righting reflexes.

Table 39. Sponsor's table of development of physical landmarks and behavioral reflexes in F1 pups.

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Groups	Control	BMY 13805-1 (mg/kg/day, p.o.) 5	27	150
Physical landmarks				
Pinnae detachment	3.9 ± 0.4 (n=11)	3.7 ± 0.7 (n=12)	3.8 ± 0.7 (n=11)	3.7 ± 0.6 (n=3)
Presence of hair	10.5 ± 0.4	10.5 ± 0.8	11.0 ± 0.6	10.7 ± 0.6
Incisor eruption	12.3 ± 0.6	12.2 ± 0.7	12.3 ± 0.3	12.5 ± 0.8
Eye opening	15.3 ± 0.5	15.2 ± 0.7	15.2 ± 0.6	14.6 ± 1.0
Testicular descent	25.0 ± 1.0	25.0 ± 1.0	25.7 ± 0.9	25.3 ± 1.0
Vaginal opening	35.0 ± 1.3	34.7 ± 2.0	35.5 ± 1.5	36.2 ± 2.9
Behavioral tests				
Surface righting	6.1 ± 0.4 (n=11)	6.4 ± 0.9 (n=12)	6.8 ± 0.9 (n=11)	7.4 ± 0.6 (n=3)
Negative geotaxis	5.9 ± 0.4	6.9 ± 0.6	7.5 ± 0.9	6.3 ± 0.5
Olfactory orientation	12.5 ± 1.1	12.0 ± 1.3	12.1 ± 1.1	12.5 ± 0.4
Auditory startle	13.5 ± 0.5	13.5 ± 0.7	13.7 ± 0.7	13.8 ± 1.2
Air righting	14.3 ± 0.8	14.2 ± 0.8	15.1 ± 1.0	15.4 ± 0.9
Visual placing	20.3 ± 1.7	20.5 ± 1.0	19.9 ± 1.2	20.8 ± 1.0

Each value shows mean ± S.E. (days). Parentheses show initial number of animals.

Learning and memory (water T-maze, with 7 blind corridors): The results (latency and errors) for males and females were combined for analysis. Group sizes varied considerably, from 22-24 for controls, LD, and MD to only 6 for HD. Acquisition proceeded similarly regardless of treatment, with average latencies for all groups of ~20 sec and errors or 1-2 after the 5th trial on the 1st day of testing (PN 25). On the second day of testing (PN26), latencies and errors were slightly higher for all groups in the 1st trial, but quickly returned to the previous performance level. When pups were tested 10 days later (PN 35), retention was considerably worse for HD pups in the first trial; latencies of 60 sec versus 20-25 for controls, LD, and MD; and errors of ~8 versus 1.5-3 for other groups). All groups performed equally well in at least 3 of the later 4 trials that day.

Motor activity of F1 offspring (4pm to 8am (light/dark 6:00/18:00), one night from PN 50-58; individually, novel cage, with food and water): Only "total activity" was measured and data presented for each hour. There were no apparent differences in activity among the different groups.

Reproductive performance of F1 offspring: It should be noted that only a small number of male and female F1 offspring from the HD F0 group were paired (6/sex versus 11-12 for the other groups). There was a suggestion of decreased fertility in the HD group; although all 6 pairs mated, only 4 of the 6 inseminated females became pregnant (i.e., 67% versus 90-100% for other groups, see table below). Looking at pregnant females, there was no treatment-related effect on length of gestation, implantation sites/dam, number of stillbirths (essentially 0), or number of live births (see table, below). However, the decreased survival index at HD (52% versus at least 80% in other groups) suggests loss of pups from this group between birth and PN 22. Calculation of the fraction of live births in each litter that were still alive on PN 4 shows an early post-natal loss of pups from the HD group (mean survival/litter was 90%, 95%, 93%, and 67% for controls, LD, MD, and HD, respectively); 3/4 litters lost 30-60% of pups by PN day 4. This effect can also be seen in the significantly decreased survival index calculated by the Sponsor (see table, below).

Table 40. Sponsor's tables showing reproductive performance of F1 offspring.

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Groups		Control	BMY 13805-1 (mg/kg/day, p.o.) 5	27	150
No. of rats mated	Male	12	12	11	6
	Female	11	12	11	6
No. of copulations	Male	12	10	10	6
	Female	11	10	10	6
% copulations/mated	Male	100	83.3	90.9	100
	Female	100	83.3	90.9	100
No. of males impregnating		12	9	10	4
% impregnatings/copulations		100	90.0	100	66.7
No. of females pregnant		11	9	10	4
% pregnants/copulations		100	90.0	100	66.7

Groups	Control	BMY 13805-1 (mg/kg/day, p.o.) 5	27	150
No. of dams	11	9	10	4
Length of gestation Mean ± S.D., day	21.8 ± 0.4	21.7 ± 0.5	21.9 ± 0.3	22.0 ± 0.0
No. of implantation sites Total	141	118	118	45
Mean ± S.D.	12.8 ± 4.0	13.1 ± 1.3	11.8 ± 3.8	11.3 ± 2.9
No. of stillborns (% per implantation sites)	0	0	1 (0.8)	0
No. of alive pups on PN1 Mean ± S.D.	12.0 ± 3.6	12.3 ± 1.6	11.1 ± 3.6	11.0 ± 2.7
Sex ratio (males/females)	1.13 (70/62)	1.27 (62/49)	0.76 (46/61)	1.32 (25/19)
Littering index	100	75.0	80.0	66.7
Implantation index	93.6	94.1	94.1	97.8
Survival index	86.6	81.9	80.0	51.6 **
Lactation index	93.4	98.3	84.5	72.7
Fertility index	109.1	84.1	91.7	66.7

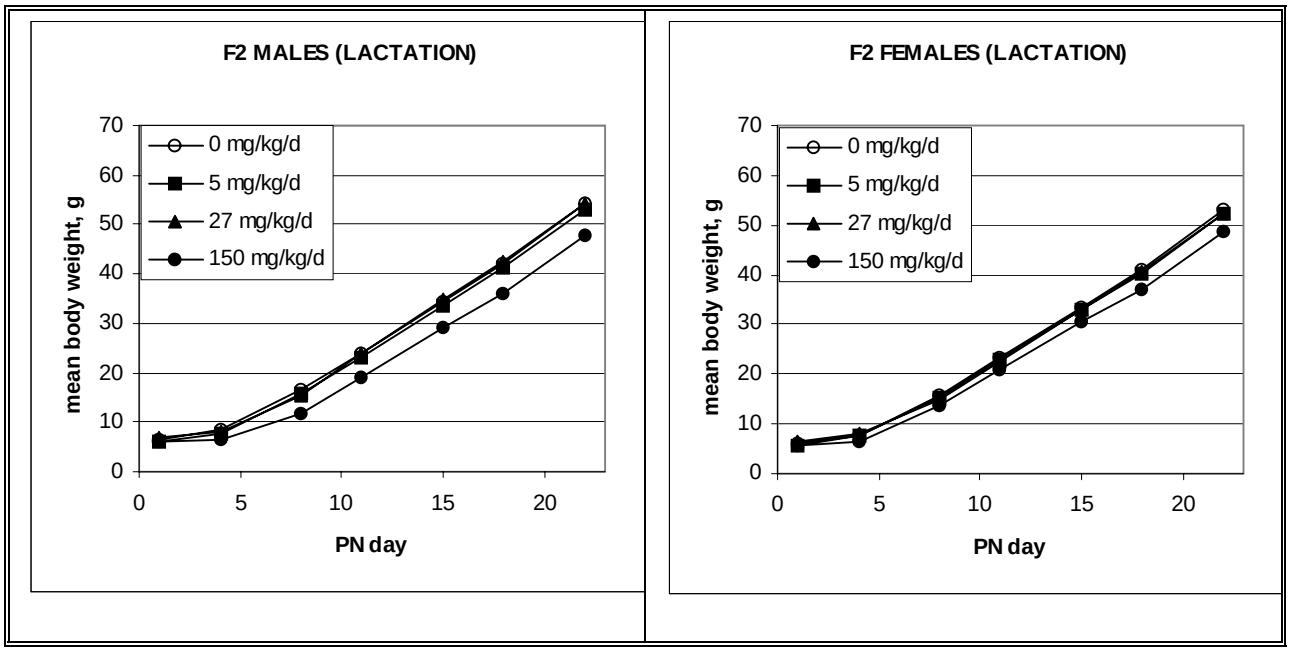
** p≤0.01 ; Significant difference from controls (Chi-square test or Fisher exact test).
Fertility index was not analyzed statistically.
Abbreviations:
Littering index - (No. live litters/No. mated) x 100
Implantation index - (Total births/Implantation sites) x 100
Survival index - (No. pups alive on Day 22/No. pups born alive) x 100
Lactation index - (No. pups alive on Day 22/No. pups alive on Day 0) x 100
Fertility index - (Total pups/No. mated) x 100/11

F2 offspring:

Post-natal mortality of F2 offspring: early post-natal mortality increased in F2 offspring of F0-drug-treated rats (see Table 40 and section above for details), however, the number of pregnancies examined was low, so the significance of this finding is unclear.

Body weights of F2 offspring: Body weights of "HD drug-treated" F2 males and females were slightly (12-28% for males, 8-14% for females) lower than controls throughout lactation to post-natal day 22 (see Figure 16, below). Apparently, these decreases did not reach statistical significance, probably because of the small sample size in this group.

Figure 16. Treatment of F0 rats with gepirone did not alter post-natal body weights of F2 pups during lactation.



Necropsy of F2 offspring: There were no remarkable effects on absolute or relative organ weights at PN day 22, although kidney weights were slightly lower (absolute ↓18%, non-significantly, and relative ↓8%, $p<0.01$, Dunnett's test) in F2 male offspring of HD F0 grandparents. There were no remarkable gross necropsy findings.

Summary of individual study findings: see key findings at beginning of this study.

2. ***Study title: Supplementary Segment I reproductive toxicity study in the rat with BMY 13805-1.***

Key study findings: Although identified by the Sponsor as a Segment I study, as with the previous study, this is actually a combined study with dosing of F0 males (prior to and through mating) and females (prior to and through mating, pregnancy, and lactation) and investigation of fertility of F0 rats and development of F1 offspring through mating, pregnancy and weaning of F2 pups.

Fertility and early embryonic development (Segment I):

- Increased latency to mating at 64 mg/kg
- No effects on number of corpora lutea, implantation sites, live or dead fetuses
- Decreased fetus weights at 64 mg/kg
- Decreased fetus lengths at 64 mg/kg

Prenatal and postnatal development including maternal (F0) function (Segment III-like):

- No effects on length of gestation (F0)
- Decreased pup birth weights at 64 mg/kg
- Decreased pup weights through weaning and to 14 weeks of age at 64 mg/kg
- Decreased food consumption after weaning at 64 mg/kg
- Tendency for increased latency to develop righting reflexes at 64 mg/kg
- No decrement in learning and memory retention trial (and no effect on acquisition) at 64 mg/kg

Other parental effects:
- Minimally decreased body weights in males at 64 mg/kg
- Decreased maternal weights at post-natal days 8-22, with decreased food consumption, at 64 mg/kg
- Effects on ovary and uterus weights of F0 females (without histopathology) that might indicate endocrine effects at 150 mg/kg.

Study no.: 0431251.2; accession no. (b) (4)-11908.
Volume #, and page #: volume 59, pages 5-155.
Conducting laboratory and location: Bristol-Myers Research Institute, Ltd., Aichi, Japan.
Date of study initiation: not specified, however, rats were received on 4/7/86 (males) and 5/26/86 (females) and were used after 1 week of acclimation.
GLP compliance: I did not find a statement, but it is the same as the previous study, which had GLP compliance statement.
QA reports: yes, see page 86 (in Japanese only in the paper copy, but translated in the electronic submission)
Drug, lot #, and % purity: BMY 13805-1; lot no 18.
Formulation/vehicle: dissolved in distilled water.

Methods:

Species/strain: male (5-week old) and female (9-week old) Sprague-Dawley rats (Crj:CD, Charles River Japan).
Doses employed: 0, 64 mg/kg/d po, for 63 days prior to mating and during mating for males, for 14 days prior to mating, during mating, and throughout gestation and lactation for females.
Route of administration: oral gavage (10 ml/kg).
Study design: half the dams (i.e., even numbers) were sacrificed near the end of gestation (day 20); the other half (i.e., odd numbers) were allowed to deliver and nurse offspring (F1), which were subsequently evaluated for physical landmarks, behavior, histopathology, and reproductive performance.
Number/sex/group: 24 F0/sex/group.
Parameters and endpoints evaluated: see previous study.

Results:

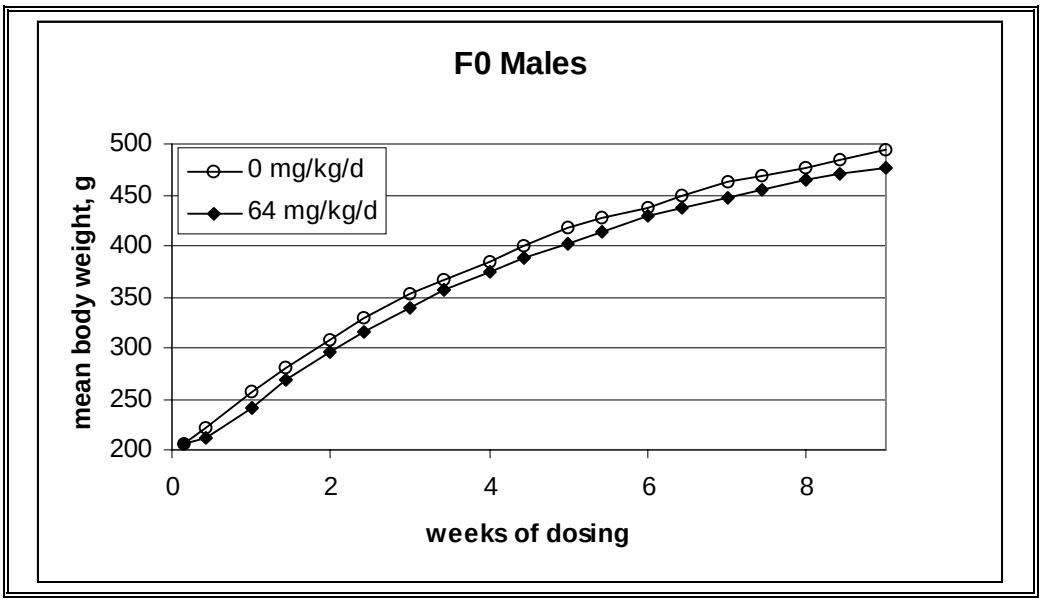
In-life observations in dams and sires (F0):

Mortality in F0 rats: Two drug-treated dams died during the study. One (#205) died at gestational day 15, due to gavage accident ("death with convulsions within [unreadable]" noted on data sheet, dosing solution in lungs noted at necropsy). Another rat (#207) was found dead on day 1 after parturition (with live pups in uterus), of unknown cause (but presumed not drug-related by the Sponsor, since there were no deaths in the previous study at more than twice this dose).

Clinical signs in F0 rats: No clinical signs related to drug-treatment were observed.

Body weight and Food consumption in F0 rats: *Pre-mating:* Mean body weights of drug-treated male F0 rats were 4-6% lower than controls throughout the 9-week pre-mating treatment period, but only significantly lower during the first 3 weeks of dosing (see Figure 17, below). Mean body weights of female rats were unaffected by the 2-week pre-mating dosing. *Gestation*: no remarkable effects. *Post-partum*: no differences in F1 dam weights through day 4 post-partum, but weights were decreased 6-9%compared with controls from day 8 through weaning at day 22. This decrease reflects the fact that control dams gained weight during this time, but drug-treated dams did not; during this time, drug-treated dams also consumed 20% less food than control dams.

Figure 17. Gepirone (64 mg/kg/d, oral gavage) slightly decreased body weights of F0 male rats during 9-week treatment before mating. [Graphed from mean values in Sponsor's summary table. NB There was an error in a value in the table that I corrected in this graph.]



Toxicokinetics: not performed.

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Mating and Fertility in F0 rats: Although all rats mated during the 3-week pairing period, drug-treated rats took longer to mate than controls (see
Figure 18, below), as was seen in the previous study. Additionally, there tended to be fewer drug-treated females that were pregnant after mating (only 78% versus 88% for controls, see Table 41,

below). There was a suggestion of increased pre-implantation loss with drug-treatment (9% versus 5% in controls, see table, below). The average litter size at day 20 of gestation was unaffected by treatment.

Table 41. Reproductive performance for all F0 dams (i.e., sacrificed at gestational day 20 or allowed to deliver at term).

PARAMETER	DOSE, mg/kg/d po	
	0	64
# of rats paired, per sex	24	24
# of matings (up to 3 wks pairing)	24	24
Matings/pairings	100%	100%
# of pregnancies	21	18
Pregnancies/matings	88%	78% [1]

[1]: 1 drug-treated female died at gestational day 15, but her pregnancy status was not reported, so this represents 18 known pregnancies out of only 23 (not 24) matings.

Figure 18. Geprione treatment (64 mg/kg/d po) decreases rate of mating in F0 rats. Values represent the cumulative number of (treated) females that were inseminated during up to 14 days of pairing with (treated) males (estimated from gestational records, volume 59, pages 96-137).

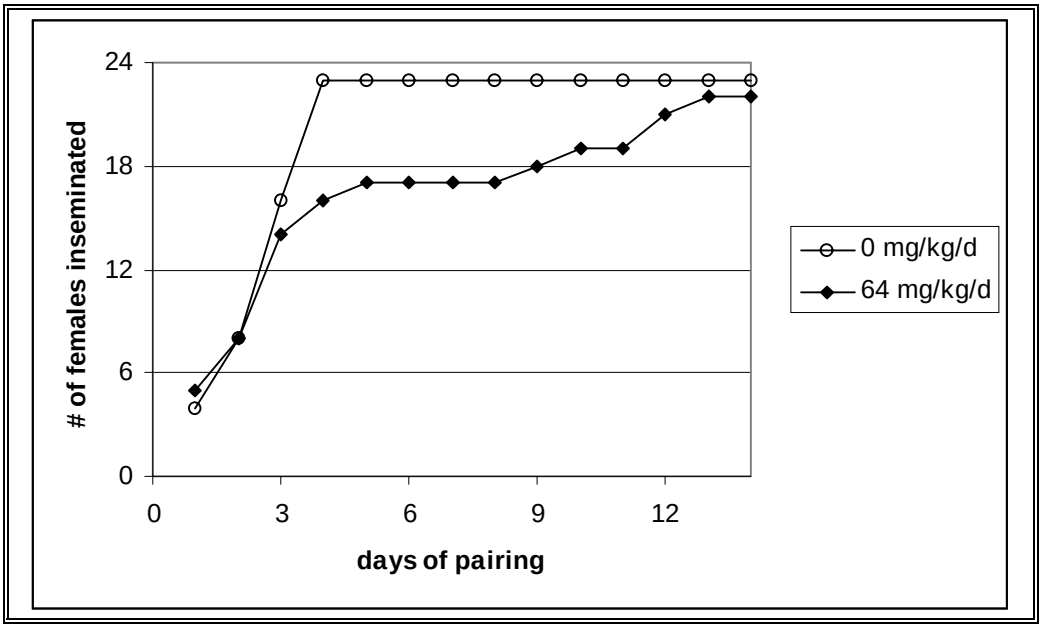


Table 42. Pre-natal development of fetuses (F0 dams sacrificed at gestational day 20). [Sponsor's values and statistics.]

PARAMETER	DOSE, mg/kg/d po	
	0	64
Non-pregnant females	2/12	3/12
# of pregnancies examined	10	9
Corpora lutea/dam (SD)	16.7 (1.6)	16.6 (2.9)
Implantations/dam (SD)	15.8 (1.6)	15.1 (1.7)
Pre-implantation loss: Mean % (SD)	5% (8.2)	9% (8.5)
Empty implantations/dam	0.7	0.4
Early resorptions/dam	0	0.3
Late resorptions/dam	0	0
Live fetuses/dam	15.1 (2.3)	14.3 (1.7)
Sex ratio, males/females	0.96	0.87
Placental weight, g	0.50	0.51
Fetal weight, g	3.67	3.28**
Fetal length, crown-rump, cm	3.67	3.52*
Fetal tail length, cm	1.46	1.52
Gross anomalies	0	0

*, **: p<0.05, p<0.01, Dunnett's test.

Table 43. Reproductive evaluation of F0 dams that delivered at term.

PARAMETER	DOSE, mg/kg/d po	
	0	64
Litters/matings	11/12	8/11
# of dams examined	11	7
Length of gestation, d	22.0	22.4
Implantation sites/dam (SD)	14.1 (2.5)	14.4 (2.4)
Total # of still births	0	11 [1]
Stillborns/implantation sites	0	10.9%
Live pups on day 1/litter	12.9 (2.8)	11.6 (5.3)
Sex ratio, males/females	1.22	1.31
Pups selected on PN day 1	85	48
Live pups on PN day 4	85	43
Live pups on PN day 22	85	43
Alive on day 4/alive on day 1	100%	90%
Alive on day 22/alive on day 4	100%	100%

[1]: These 11 still births include 9 from a single litter and 1 each from 2 other litters.

Necropsy of F0 rats: *Sires (after mating):* decreases in absolute weights of several organs, that normalized when corrected for decreased body weights; increases in absolute (and relative) weights of lungs (absolute ↑6%, relative ↑14% versus controls) and adrenals (absolute ↑9%, relative ↑18% versus controls). *Dams (at term):* notable 20% increase in absolute and relative mean ovary weight. *Dams (after weaning):* notable increase in mean pituitary weight (absolute ↑12%, relative ↑21% versus controls); ~30% decrease in absolute and relative mean thymus weight; decrease in absolute kidney weight (↓12%) that didn't completely normalize when corrected for body weight (↓6%). Gross necropsy findings were minimal and largely limited to

findings of congestion in several organs in 2 females that died prematurely (1 with dosing solution in lungs, confirming the dosing accident as cause of death); 1 dosed dam with all pups dead.

In-life observations, F1 offspring:

Post-natal survival of F1 offspring: There were no differences in post-natal survival for F1 pups from control and drug-treated F0 parents (see Table 44, below).

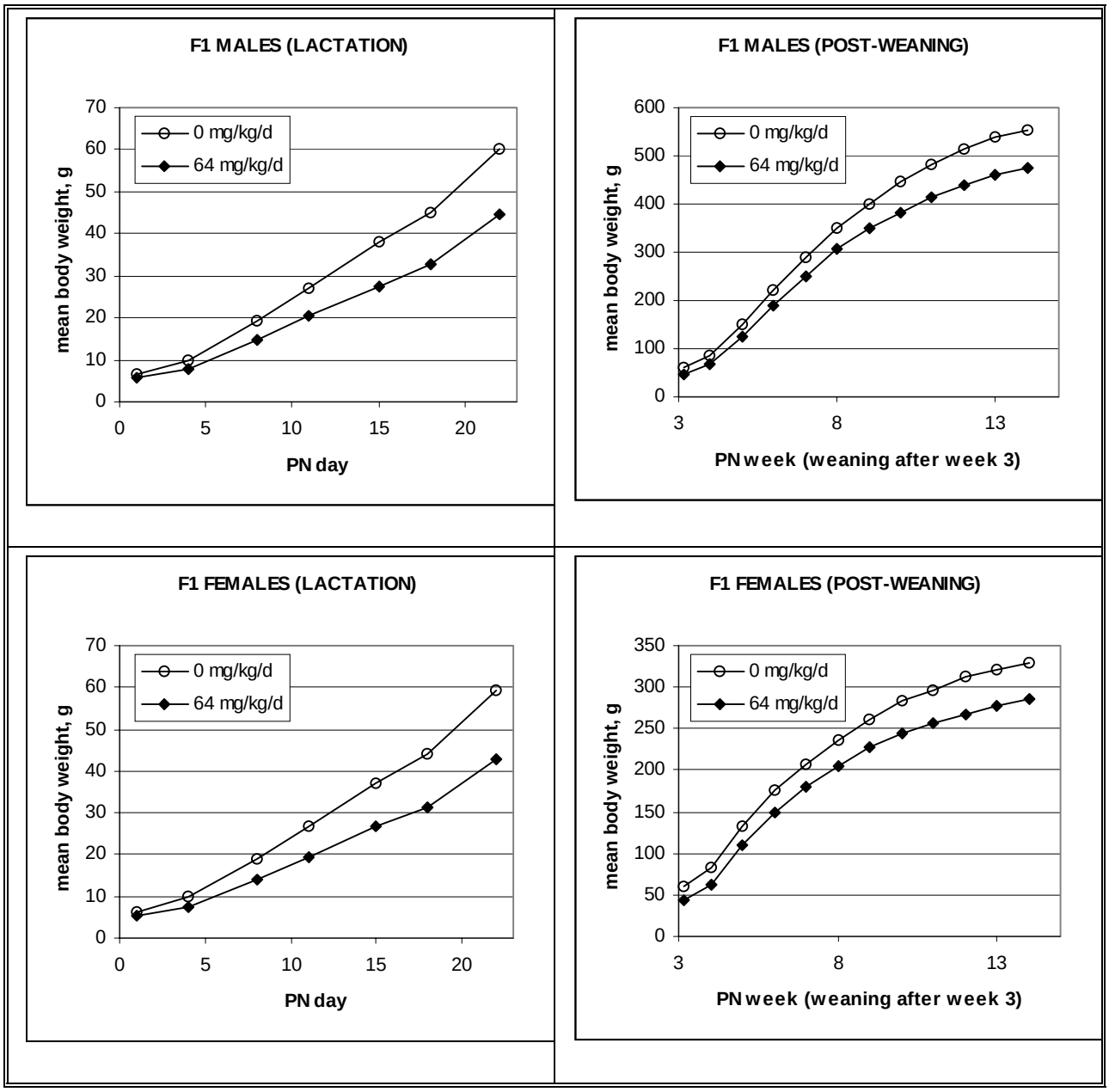
Table 44. Post-natal survival of all F1 pups that were alive on post-natal day 1, not just "selected pups" as in the Sponsor's table above.

F1 PARAMETER	F0 DOSE, mg/kg/d po	
	0	64
Total litters with pups alive on PN day 1	11	6
Total pups alive on PN day 1, M+F	77+64=141	43+32=75
Total pups alive on PN day 4, M+F	43+42=85	26+17=43
Total pups alive on PN day 22, M+F	43+42=85	26+17=43
PND4/PND1, %, M, F→Total	56, 66→60%	60, 53→57%
PND22/PND4, %, M, F→Total	100, 100→100%	100, 100→100%
PND22/PND1, %, M, F→Total	56, 66→60%	60, 53→57%
Mean live pup weights, g, PN day 1, M,F	6.54, 6.26	5.72**, 5.32**

**: p<0.01, versus controls, Dunnett's test.

Body weights and food consumption of F1 offspring: Body weights of F1 pups from gepirone-treated dams and sires (F0) had lower body weights at birth (~15% lower than controls, see table above), during lactation (~25% lower than controls), and after weaning until at least 14 weeks of age (~15% lower than controls throughout this period) (see Figure 19, below). These decreases in body weight reflected decreased food consumption; an average of 2-5 g less for males and 1-4 g less for females after weaning.

Figure 19. Geprione (64 mg/kg/d) treatment of F0 generation (sires and dams) results in decreased body weights of male and female F1 pups, at birth, during lactation, and to at least 14 weeks of age.



Physical landmarks and behavioral reflexes of F1 offspring: Although no significant differences were noted in the report, the presence of hair and the eruption of incisors was delayed 1 day in the offspring of gepirone-treated parents (see Table 45, below). Righting reflexes were also slightly (but not statistically) delayed (air righting by 1 day and surface righting by 0.8 day).

Table 45. The Sponsor's table showing physical development and behavioral reflexes of pups (F1). Values represent the day when 50% of each dose-group tested positive for each trait.

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Groups	Control	64 mg/kg/day
No. of dams	11	6
Pinnae detachment	3.3 ± 0.3	3.8 ± 0.3
Presence of hair	10.0 ± 0.0	11.0 ± 0.1
Incisor eruptions	11.3 ± 0.6	12.4 ± 0.6
Eye opening	14.8 ± 0.3	14.7 ± 0.5
Testicular descent	26.3 ± 1.0	26.0 ± 1.5
Vaginal opening	33.5 ± 1.4	33.6 ± 1.4
Each value shows mean ± S.D. (days).		

Groups	Control	64 mg/kg/day
No of dams	11	6
Surface righting	6.5 ± 0.6	7.3 ± 0.7
Negative geotaxis	7.5 ± 0.6	8.2 ± 0.4
Olfactory orientation	11.4 ± 0.9	11.6 ± 1.2
Auditory startle	13.1 ± 0.5	13.7 ± 0.7
Air righting	15.8 ± 0.6	16.8 ± 0.5
Visual placing	21.3 ± 0.3	21.4 ± 1.0
Each value shows mean ± S.D. (days).		

Learning and memory (water T-maze, with 7 blind corridors) of F1 offspring: No treatment-related effects. The results (latency and errors) for males and females were combined for analysis. Group sizes varied considerably, from 22 for controls to only 12 for drug-treated. Acquisition proceeded similarly regardless of treatment, with average latencies for both groups of ~20 sec and errors of ~1-2 after the 5th trial on the 2nd day of testing (PN 26). When pups were tested 10 days later (PN 35), retention was similar for both groups; with latencies of ~30 sec and errors of ~3-4 in the 1st trial. Both groups improved similarly in the remaining 4 trials that day.

Motor activity of F1 offspring (4pm to 8am (light/dark 6:00/18:00), one night from PN 50-58; individually, novel cage, with food and water): Only "total activity" was measured and data presented for each hour. There were no apparent differences in activity among the different groups.

Reproductive performance of F1 offspring: It should be noted that only a small number of male and female F1 offspring from the drug-treated F0 group were paired (6/sex versus 11 for controls). There was no decrease in fertility in the drug-treated group; all 6 pairs mated and all of the 6 inseminated females became pregnant (i.e., 100% versus 80% for controls, see table below). Looking at pregnant females, there was no treatment-related effect on length of gestation or number of stillbirths (essentially 0). The number of implantation sites/dam was slightly (but non-significantly) increased (44% versus controls) and the number of live births was similarly increased (significantly, 47% versus controls, see Table 46, below).

The decreased survival index for drug-treated pups (69% versus 94% for controls, see Table 46, below) suggests loss of pups from this group between birth and PN 22. The Sponsor tabulated the number of surviving pups at days 1, 4 and 22 (see table, below), however, their values are based upon a subset of pups that were alive on PN day 1. I have calculated the post-natal survival based upon all pups that were alive on day 1, with sex differences noted (see table, below). In general, female pups survived slightly better than males regardless of group. For controls, there was an overall 30% loss of pups between days 1 and 22, with essentially all of that loss occurring between days 1 and 4 (27%). The F2 offspring from F0 dams that had been drug-treated through lactation survived considerably less well than controls, with an overall 60% loss between days 1 and 22 and most of that (50%) between days 1 and 4.

Table 46. Sponsor's tables showing reproductive performance of F1 offspring.

Groups	Control	64 mg/kg/day
No. of pairs mated	11	6
No. of copulations	10	6
% copulations/mated	90.9	100
No. of males impregnating	8	6
% impregnatings/copulations	80.0	100
No. of females pregnant	8	6
% pregnants/copulations	80.0	100

Groups		Control	64 mg/kg/day
No. of dams		8	6
Length of gestation	Mean ± S.D., day	22.0 ± 0.5	22.2 ± 0.4
No. of implantation sites	Total	78	84
	Mean ± S.D.	9.8 ± 5.1	14.1 ± 1.7
No. of stillborn pups (% per implantation sites)		1 (1.3)	1 (1.2)
No. of alive pups on Day PN1	Total	74	82
	Mean ± S.D.	9.3 ± 4.7	13.7 ± 1.4*
Sex ratio (males/females)		1.31 (42/32)	1.10 (43/39)
No. of alive pups selected on Day PN1		55	48
No. of alive pups on Day PN4		54	40
No. of alive pups on Day PN22		52	33
Littering index		72.7	100
Implantation index		94.9	97.6
Survival index		94.5	68.8##
Lactation index		96.3	82.5#
Fertility index		61.2	124.2

* p<0.05: Significant difference from controls (Multiple comparison method of Dunnett).
# p<0.05, ## p<0.01: Significant difference from controls (Chi-square test or Fisher exact test).
Fertility index was not analyzed statistically.

Table 47. Post-natal survival of all F2 pups that were alive on post-natal day 1, not just "selected pups" as in the Sponsor's table above.

F2 PARAMETER	F0 DOSE, mg/kg/d po	
	0	64
Total litters with pups alive on PN day 1	8	6
Total pups alive on PN day 1, M+F	42+32=74	43+39=82
Total pups alive on PN day 4, M+F	29+25=54	19+21=40
Total pups alive on PN day 22, M+F	27+25=52	14+19=33
PND4/PND1, %, M, F→Total	69, 78→73%	44, 54→49%
PND22/PND4, %, M, F→Total	93, 100→96%	74, 90→82%
PND22/PND1, %, M, F→Total	64, 78→70%	33, 49→40%
Mean live pup weights, PN day 1, M,F	7.07, 6.70	6.25 *, 5.98

*: p<0.05, versus controls, Dunnett's test.

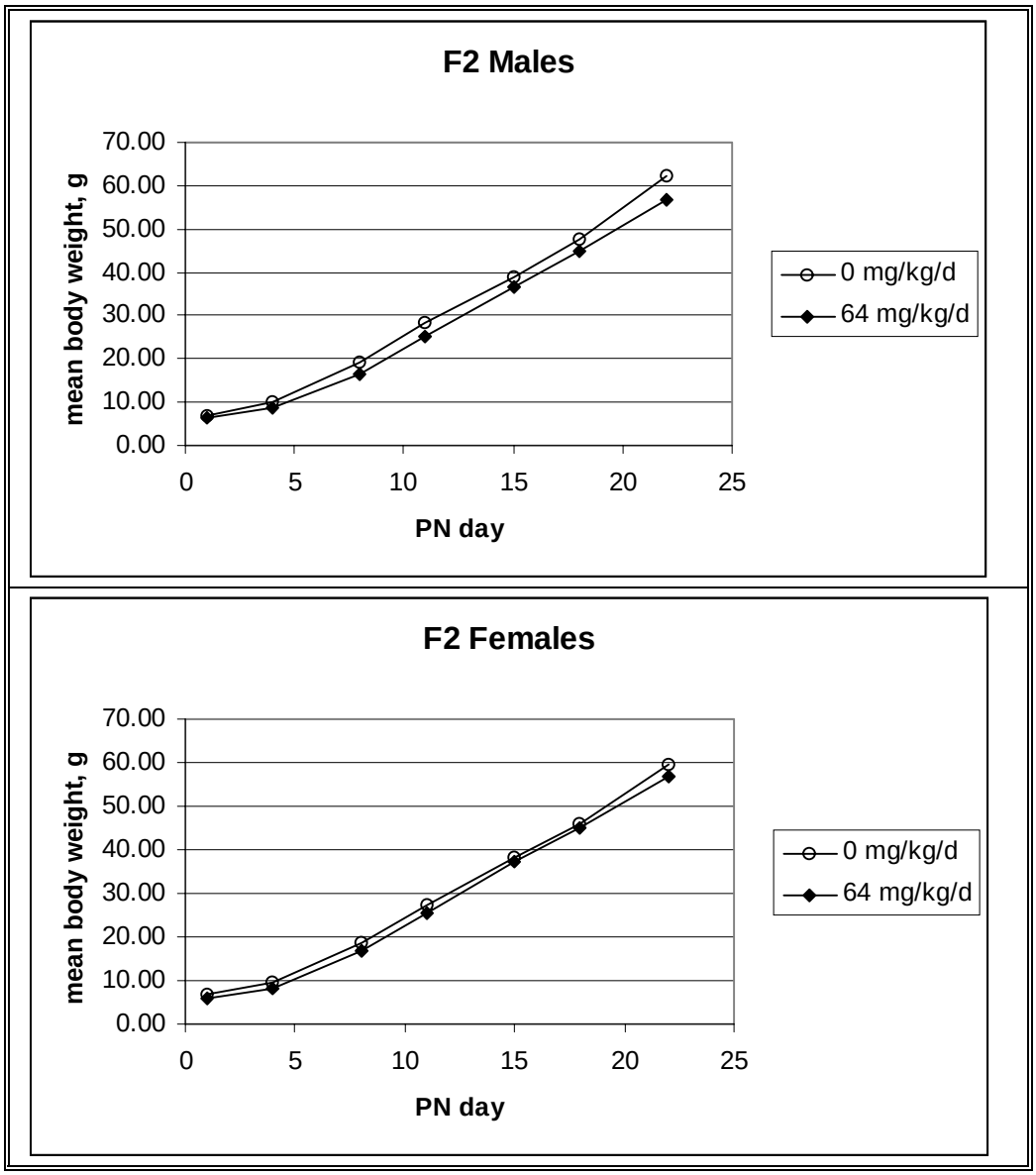
Necropsy of F1 offspring (some at weeks 10 and 14, and after mating): The absolute weights of several organs were decreased in drug-treated offspring, but essentially all normalized when corrected for the decreased body weights in this group. Gross findings were minimal and limited to a hemorrhage in the thymus of 1/6 drug-treated F1 females at 14 weeks and dilated renal pelvis in a few controls (3/11 males and 1/11 females at 14 weeks).

F2 offspring:

Post-natal mortality: early post-natal mortality was increased in F2 offspring of F0-drug-treated rats (see table and section above for details), however, the number of pregnancies examined was low, so the significance of this finding is unclear.

Body weights: Although body weights of "drug-treated" F2 males were slightly (12%), but significantly lower than controls on post-natal day 1, there were no differences at later time points up to post-natal day 22 (see Figure 20, below).

Figure 20. Treatment of F0 rats with gepirone (64 mg/kg/d) did not alter post-natal body weights of F2 pups.



Necropsy: There were no remarkable effects on absolute or relative organ weights at PN day 22. The only necropsy finding that was noted was atrophy of testes in 1/14 males from drug-treated F0 grandparents (versus 0/27 controls).

Summary of individual study findings: See key findings listed at the beginning of the study.

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

1. *Study title: A segment II reproduction study with orally administered MJ 13805-1 in rats.*

Key study findings:

Embryo-fetal variables:
- No effects on embryo/fetal death
- Decreased fetal weights at 150 and 300 mg/kg
- Decreased fetal length at 300 mg/kg
- No major anomalies
- Increased common skeletal variations, at 150 and esp 300 mg/kg

Maternal variables:
- Decreased food consumption at all doses
- Decreased body weights, esp at 300 gm/kg

Study no.: 628; accession no. (b) (4)-11425 (electronic version of submission).
Volume #, and page #: volume 56, pages 5-83.
Conducting laboratory and location: Bristol-Myers Co., Montpellier, France.
Date of study initiation: first day of mating, 2/18/85 (to last day of sacrifice, 3/21/85).
GLP compliance: yes, see page 9.
QA reports: yes, see page 38.
Drug, lot #, and % purity: MJ-13805-1, lot no. 15, ref E 84G 031,
Formulation/vehicle: suspension in 0.5% carboxymethyl cellulose in deionized water; dosing suspensions prepared daily, immediately before administration, MD and LD by dilution of HD suspension.

Methods:

Species/strain: male and female Sprague-Dawley CD rats, sexually mature ((b) (4)).
Doses employed: 0, 75, 150, and 300 mg/kg/d (10 ml/kg); based upon acute, 4-week and 13-week toxicity studies in rats and teratology study in rabbits.
Route of administration: oral gavage (10 ml/kg/d), once a day in the morning on days 6-15 of pregnancy.
Study design: virgin females (210-280 g) were mated with males (1 male and 2 females per cage); day of mating (i.e., appearance of copulatory plug) was designated day 0 of pregnancy and all inseminated females were randomized to treatment groups and housed individually (mesh-bottom polypropylene cages with food and water *ad libitum*).
Number/sex/group: 25 inseminated females per group.
Parameters and endpoints evaluated: *Maternal variables in life*: body weights on days 0, 6, 12, 15, and 21 of gestation; food consumption weekly; appearance and behavior daily.

At necropsy on day 21 of gestation the following parameters were recorded (quoting from the report):

"

a. Number of early resorptions (details of definable placentas, but no recognizable fetuses).

b. Number of late resorptions (recognizable fetuses undergoing resorption).

c. Number and identification of dead fetuses (no signs of movement, but not in a state of visible degeneration).

d. Number and identification of live fetuses (spontaneous movement).

e. Number of empty implantation sites (evidence of regressing cotyledons, no evidence of placenta).

f. Description and identification of any malformed fetuses or uterine abnormalities.

g. Individual body weights and crown-rump distances.

h. Sex of fetuses.

Approximately 50 % of the total pups from each litter were eviscerated, cleared and stained with Alizarin Red S for examination of skeletal anomalies. The remaining animals were preserved in Bouin's solution and examined by method of Wilson for evidence of soft tissue anomalies.

"

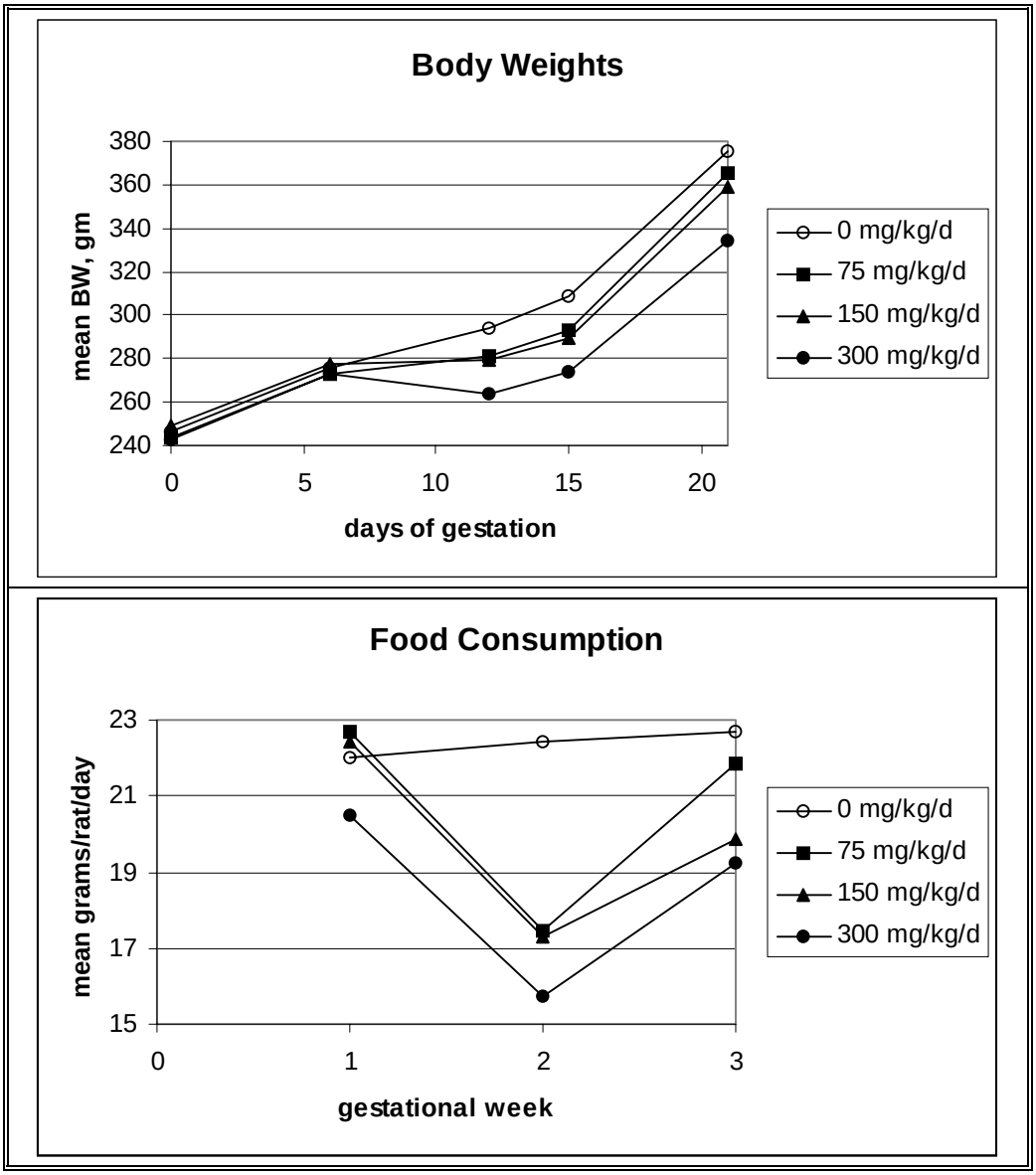
Results:

Mortality of F0 dams: 2/25 HDF (i.e., 300 mg/kg/d) died during dosing period: 1 found dead just before 4th dosing, a size enlargement of the vessels of meninges was found at necropsy; the other died 15 min after the 3rd administration, following hypoactivity and clonic convulsions, with an enlargement of the vessels of meninges noted at necropsy. This gross finding of enlargement of meningeal vessels noted for both pregnant dams that died after 3 or 4 daily doses of 300 gm/kg is intriguing, but was not noted in other general toxicology studies in rats at similar doses. The acute toxicity (LD50) studies could have provided relevant information, since very high oral and ip doses were examined and there were relatively many deaths preceded by convulsions, however, necropsies were not performed.

Clinical signs in F0 dams: hypoactivity for all HD dams; no signs noticed at lower doses.

Body weight and food consumption of F0 dams during gestation (see Figure 21, below): Body weight gains showed dose-related decreases during dosing, apparent from GD12-15. However, body weight gains between GD15 and GD22 were similar for all groups, although body weights of drug-treated dams remained slightly decreased at GD22, especially at HD (~10% lower than controls at GD22). Decreases in food consumption paralleled the decreases in body weights in drug-treated groups.

Figure 21. Body weights and food consumption of F0 dams during gestation. [NB The scales for body weights and food consumption are expanded; the magnitude of differences between groups appear exaggerated.]



Toxicokinetics: not performed.

Terminal and necroscopic evaluations:

Dams:

Table 48. Maternal variables for Segment II study of 0, 75, 150, and 300 mg/kg oral (gavage) doses of gepirone in rats. [Sponsor's values.]

PARAMETER	DOSE, mg/kg/d (gestational D6-15)			
	0	75	150	300
Total pregnancy rate	23/25	25/25	24/25	24/25
Mortalities	0	0	0	2
Pregnant Females at termination	23	23	24	22
Mean implantation sites	13.83	13.22	14.25	13.95
Mean corpora lutea	15.30	15.09	15.92	15.32
Mean live fetuses	13.30	12.70	13.67	13.09
Mean dead fetuses	0	0	0	0
Mean early resorptions	0.00	0.00	0.00	0.18
Mean late resorptions	0.00	0.09	0.04	0.09

F1 offspring: Drug treatment did not affect the number of live fetuses per litter, however, the fetuses at MD and especially HD were smaller than controls (see table below). The average fetal body weight was decreased 14% at MD and 27% at HD; and the average crown-to-rump length was decreased 9% at HD; the Sponsor recognized this effect, but did not perform statistical analysis on the data. Approximately half of each litter was examined for visceral abnormalities and the other half for skeletal abnormalities; between 139 and 169 fetuses were examined for each class of abnormality.

Visceral abnormalities were rare (all are displayed in Table 49, below); the only abnormalities that were more prevalent in drug-treated fetuses were subcutaneous hematoma of the trunk in 2 fetuses from a single HD litter, not seen in any other treatments, a single incidence of lack of ventricular septum in the heart of 1 LD fetus, and slightly increased incidence of dilated pelvis of the kidney at LD. None of these visceral abnormalities were attributable to drug treatment.

In contrast, drug-treated fetuses, especially at HD, showed increased incidence of many common skeletal variations (i.e., incomplete ossifications of skull, vertebrae and sternebrae, missing sternebrae, and shorter ribs; see Table 49, below). The Sponsor notes that these skeletal abnormalities correlate with the decreased body weight and length of fetuses at HD. This Reviewer agrees that the fetuses in the HD group seem to be slightly less developed than controls, without showing any major abnormalities.

Table 49. Embryo/fetal variables for Segment II study of 0, 75, 150, and 300 mg/kg oral (gavage) doses of gepirone in rats. [Sponsor's values.]

PARAMETER	DOSE, mg/kg/d (gestational D6-15)			
	0	**75**	**150**	**300**
Mean live fetuses/litter	13.30	12.70	13.67	13.09
Mean fetus weights, g	**5.04**	**4.88**	**4.32**	**3.70**
Mean fetus length, mm	**40.13**	**39.77**	**38.56**	**36.60**
Total litters examined	23	23	24	22
Total fetuses examined for visceral abn	147	142	159	139
Trunk: subcutaneous hematoma	0	0	0	2 (1 litter)
Heart: lack of ventricular septum	0	1 (1 litter)	0	0
Kidneys: enlarged pyelic cavity	3 (3 litters)	6 (5 litters)	2 (1 litter)	1 (1 litter)
Tale: twisted	0	0	0	1 (1 litter)
Total fetuses examined for skeletal abn	159	150	169	149
Incomplete ossifications:				
Skull, frontal	13 (5 litters)	8 (3 litters)	11 (3 litters)	26 (11 litters)
Skull, parietal	17 (6 litters)	8 (3 litters)	12 (4 litters)	29 (12 litters)
Skull, interparietal	1 (1 litter)	0	0	3 (3 litters)
Vertebrae, thoracic, 10th	0	0	1 (1 litter)	6 (3 litters)
Vertebrae, thoracic, 11th	1 (1 litter)	2 (1 litter)	2 (1 litter)	7 (5 litters)
Vertebrae, thoracic, 10-13th	0	0	0	4 (2 litters)
Vertebrae, caudal	1 (1litter)	0	0	9 (5 litters)
Thorax, sternabrae, 2nd	2 (2 litters)	4 (3 litters)	16 (8 litters)	35 (14 litters)
Thorax, sternabrae, 4th	0	1 (1 litter)	0	13 (8 litters)
Thorax, sternabrae, 6th	0	0	0	12 (9 litters)
Forefeet, metacarpal + distal phalanges	0	0	0	5 (2litters)
Shorter ribs (thoracic)				
13^{th}, unilateral	1(1 litter)	2 (2 litters)	1 (1 litter)	6 (4 litters)
13^{th}, bilateral	2 (2 litters)	3 (2 litters)	5 (4 litters)	7 (5 litters)
14^{th} rib starting unilateral	0	0	0	5 (3 litters)
5^{th} sternebrae missing	9 (7 litters)	4 (3 litters)	18 (8 litters)	46 (15 litters)
6^{th} sternebrae missing	0	0	0	27 (11 litters)

Summary of individual study findings: see key findings at beginning of study.

2. *Study title: A Segment II reproduction toxicity study in rabbits with MJ 13805-1.*

Key study findings:

Embryo-fetal variables:
- No effects on embryo/fetal death
- Decreased fetal weights at 100 and 200 mg/kg
- Decreased fetal length at 100 and 200 mg/kg
- No major anomalies
- Increased common skeletal variations, at 200 mg/kg

Maternal variables:
- Decreased body weights at 100 and 200 gm/kg
- Food consumption was not measured

Study no.: 13805-401-42-83; accession no. (b) (4)-10011 (designates electronic submission).
Volume #, and page #: volume 56, pages 207-266.
Conducting laboratory and location: Bristol-Myers Co., Mt. Vernon, IN.
Date of study initiation: 7/19//83; terminated on 8/18/83.
GLP compliance: yes, see page 209.
QA reports: yes, see page 241.
Drug, lot #, and % purity: MJ 13805-1, lot #5, purity data not provided, however, elemental, IR, NMR, and MS analysis results were consistent with the assigned structure (9/20/83 report).
Formulation/vehicle: dissolved in sterile water; all solutions were prepared once, at the start of dosing, and refrigerated when not in use; samples of drug dosing solutions from the day of preparation were analyzed and were 100-102% of nominal concentrations; Sponsor states that stability had been previously determined in study 13805-002-15-83.

Methods:

Species/strain: sexually mature New Zealand White rabbits, 5-6 months old, weighing 3.9-4.0 kg, from (b) (4). Does were induced to ovulate (with pituitary LH) and artificially inseminated with ~3 million viable sperm from 1 of 3 proven male breeders. Half (9 does) of each main study group was inseminated with sperm from buck #1 on day 1; the other half (9 does) with sperm from buck #2 on day 2; and the satellite groups (5 does each) with sperm from buck #3 on day 3.
Housing Conditions: individually, with food and water *ad libitum*.
Doses employed: 0, 50, 100, and 200 mg/kg/day (1.0 ml/kg) by oral gavage on days 6-18 of gestation (i.e., days after insemination). Doses were based upon a range-finding study.
Route of administration: oral gavage.
Study design: Dams were dosed from GD 6 through 18; dams were sacrificed on GD 29; live fetuses were incubated *in vitro* and survival at 6 and 24 hr was determined; fetuses were then necropsied for visceral (half) or skeletal (other half) anomalies and variations.
Number/sex/group: 18 inseminated females per main study dose group; 5 additional at 50 and 200 mg/kg/d for ???

Parameters and endpoints evaluated: Quoting from the Sponsor, the following observations were recorded:

"

a. Number of early resorptions (details of placenta definable but no recognizable fetus).

b. Number of late resorptions (recognizable fetus undergoing resorption).

c. Number and identification of dead fetuses (no signs of movement, but not in a state of visible degeneration).

d. Number and identification of live fetuses (spontaneous movement).

e. Number of empty implantation sites (evidence of regressing cotyledons, no evidence of placenta).

f. Description and identification of any malformed fetuses or uterine abnormalities.

g. Individual body weights and crown-rump distances.

Following the examination of the fetuses and uterine contents, each litter of live pups was placed in an incubator and survival was determined at 6 and 24 hours. At the end of the 24 hour observation period, the neonates were sacrificed (0.1 ml of T-61® injected intraperitoneally), crown-rump distances measured and a visceral examination[1] was performed, which consisted of evaluating all cavitated organs, excluding the brain. All pups were then skinned and stained with Alizarian Red S by a modified method of Green[2] for examination of skeletal anomalies. "

Results:

Mortality: Several does died during the dosing period (see Table 50, below). Several of the deaths were attributable to gavage accidents, but the cause of death was not determined for others. It is possible, though not clear, that the higher incidence of deaths at the HD (22% versus 11% in controls and MD and 0% at LD) reflects direct toxicity of the drug.

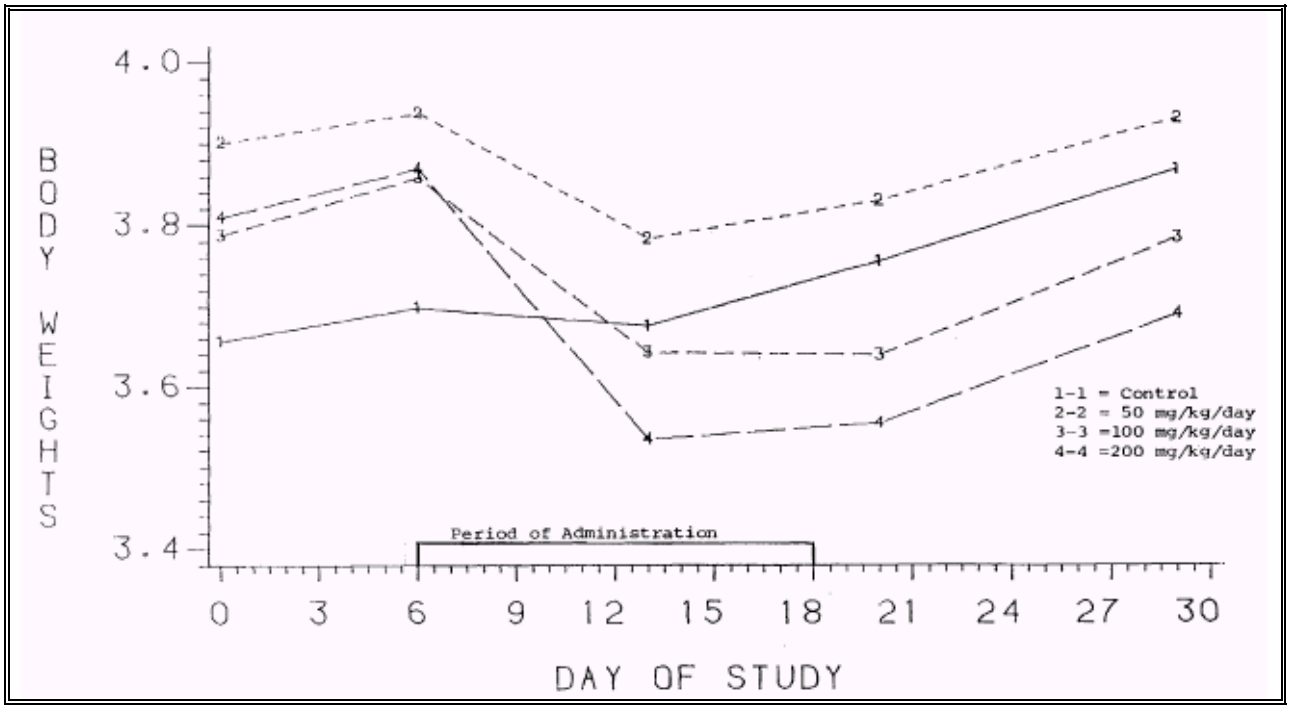
Table 50. Deaths of F0 does during the study.

DOSE	DEATHS	DAY(S) OF DEATH(S)-GESTATIONAL
0	2/18 = 11%	12 (pregnant, cause not determined) 14 (not pregnant, gavage accident)
50	0/18 = 0%	
100	2/18 = 11%	13 (not pregnant, fluid aspiration) 17 (pregnant, cause not determined)
200	4/18 = 22%	8 (not pregnant, cause not determined) 11 (not pregnant, cause not determined) 10 (pregnant, lung dark areas) 18 (pregnant, fluid aspiration)

Clinical signs: not mentioned.

Body weights of F0 dams: Body weights of drug-treated does showed dose-related decreases during dosing: GD 13 weights were 99%, 96%, 94%, and 91% of GD 6 weights in controls, LD, MD, and HD groups, respectively (see Figure 20, below). After the termination of dosing (i.e., between GD 20 and 29), all groups appeared to gain weight at similar rates. However, drug-treated does gained less weight during pregnancy than controls (GD 29 weights were 106%, 101%, 100%, and 97% of GD 0 weights in controls, LD, MD, and HD groups, respectively).

Figure 22. Maternal body weights were decreased by gepirone treatment. [Sponsor's graph of body weights (kg) of does.]



Food consumption: not measured.

Toxicokinetics: not performed (or at least I did not find results mentioned here).

Terminal and necroscopic evaluations of dams: The Sponsor did not note any changes in maternal variables reflecting changes in reproductive performance due to drug administration. However, the total pregnancy rate appeared lower at the high dose (11/18 versus 14-16/18 in other groups) though this was not statistically significant (see table, below).

Table 51. Maternal variables for Segment II study of 0, 50, 100, and 200 mg/kg oral (gavage) doses of gepirone in rabbits.

PARAMETER	DOSE, mg/kg/d (gestational D6-18)			
	0	50	100	200
Total pregnancy rate	**16/18 88.9%**	**14/18 77.8%**	**15/18 83.3%**	**11/18 61.1%**
Mortalities	2/18	0/18	2/18	4/18
Pregnant Females at termination	**15/16**	**14/18**	**14/16**	**9/14**
Pregnant females with live fetuses	**13/15 86.6%**	**13/14 92.9%**	**11/14 78.6%**	**8/9 88.8%**
Mean implantation sites	6.38	8.00	6.93	**8.73**
Mean live fetuses	**4.60**	**6.50**	**5.50**	**6.33**
Mean dead fetuses	**0**	**0.07+**	**0**	**0**
Mean early resorptions (including empty sites	1.47	1.29	1.21	2.33
Mean late resorptions	0.13	0.14	0.71	0
Total post-implantation loss, %	24/102 24%	20/112 17%	18/104 17%	21/96 22%

+: represents 1/10 fetuses in a single litter.

Terminal and necroscopic evaluations of F1 fetuses: There were no notable findings in terms of live fetuses per litter, dead fetuses per litter (essentially none), or post-implantation loss (see table, above). However, F1 fetuses from HD does were significantly smaller than controls, averaging 20% lower body weight and 13% shorter crown-rump length. F1 fetuses from MD does were also significantly shorter, averaging 6% shorter than controls, correlated with a (non-significant) 13% decrease in average body weight. The total number of fetuses examined for anomalies and variations was low (less than 100 in each dose group for each class of aberrations, *viz*., visceral and skeletal); this would limit the detection of rare malformations. Only a single fetus (1/9 in a MD litter) showed any anomalies and this fetus had several skeletal and one visceral anomaly (see footnote to Table 52, below, for details). Only increases in the incidence of some skeletal variations appeared to be drug-related: F1 fetuses from HD does had increased incidence of (bilateral) supernumerary 13th rib (35% versus 14% in controls); F1 fetuses from all drug-treated groups had increased incidence of the combination of rudimentary and supernumerary 13th rib (60-66% versus 42% in controls). Visceral variations were sporadic and not treatment-related.

Table 52. Sponsor's table of embryo/fetal variables for Segment II study of 0, 50, 100, and 200 mg/kg oral (gavage) doses of gepirone in rabbits.

Group	HA1F	HA2F	HA3F	HA4F
Compound (MJ)	Vehicle	13805-1	13805-1	13805-1
Dose (mg/kg/day)	0.0	50.0	100.0	200.0
Total Grossly Normal Fetuses/Number Examined	69/69	92/92	76/77	57/57
Mean Fetus Weight (g)	42.8	39.6	37.2	34.4*
Mean Crown-Rump Distance (cm)	9.4	9.1	8.8**	8.2*
Skeletal Anomalies/ Number Examined			+	
-Talipes (hindfeet)	0/69	0/92	1/77	0/57
Skeletal Variations/ Number Examined				
-Rudimentary 13th Rib (Bil)	15/16	21/92	29/77	6/57
-Rudimentary 13th Rib (Lt)	4/69	8/92	7/77	6/57
-Rudimentary 13th Rib (Rt)	0/69	7/92	5/77	5/57
-Supernumerary 13th Rib (Bil)	10/69	19/92	10/77	20/57**
-Rudimentary, Supernumerary 13th Rib (Comb)	29/69	55/92**	51/77*	37/57**
-Unossified 5th Sternebra	10/69	6/92	6/77	2/57
-Inc. Ossification of Sternebra	5/69	6/92	7/77	1/57
-Fused Sternebrae	0/69	2/92	1/77	0/57
-Bifurated Ribs (5th & 6th)	1/69	0/92	1/77	0/57
-Bifurated Ribs (8th & 9th)	0/69	1/92	0/77	0/57
-Bilobed Thoracic Vertebrae (4th & 5th)	0/69	0/92	1/77	0/57
-10 Ribs (Lt)	0/69	0/92	1/77	0/57
-Floating 8th Rib	0/69	0/92	1/77	0/57
Visceral Anomalies/ Number Examined	0/69	0/92	1/77	0/57
Visceral Variations/ Number Examined				
-Mottled Lungs	6/69	7/92	0/77	1/57
-Pale Kidney (Rt)	1/69	0/92	0/77	0/57
-Pale Kidney (Lt)	1/69	0/92	0/77	0/57
-Pale Kidney (Bil)	0/69	1/92	0/77	0/57
-Dark Thymus	0/69	1/92	0/77	0/57
-Lungs Mottled & Red	1/69	0/92	0/77	0/57

* = P ≦ 0.01 Significance Level (Chi-square test)
** = P ≦ 0.05 Significance Level (Chi-square test)

+One pup in a litter (HE3F-05) of 9 had talipes equinovarus, a deformity of the foot in which both hindfeet in this pup were turned downward and inward. Skeletal observations confirmed the talipes. Other skeletal anomalies/variations for this animal were fused 3rd and 4th sternebrae, bilobed 4th & 5th thoracic vertebrae, unossified 5th sternebra, 10 ribs on the left rib cage, bifurated 5th and 6th ribs and a floating 8th rib. Visceral examinations showed the left kidney of this animal had coalesced with the right kidney. Each kidney had a separate ureter extending into the urinary bladder.

Survival of F1 29-day fetuses incubated *in vitro* for 24 hr after delivery (see Table 53, below): There was no drug-related decrease in *in vitro* viability measured after 6 or 24 hr. Survival for 24 hr was high (81-88%) for all groups.

Table 53. Sponsor's table showing *in vitro* survival of F1 pups.

Group	HA1F	HA2F	HA3F	HA4F
Number Incubated	69	91	77	57
Alive at 6 Hours	65 (94%)	85 (93%)	65 (84%)	52 (91%)
Alive at 24 Hours	60 (87%)	77 (85%)	62 (81%)	50 (88%)

Summary of individual study findings: see key findings at the beginning of this study.

C. Study title: A Segment III reproductive toxicology study with orally administered BMY 13805-1 in rats.

Key study findings:

Prenatal and postnatal development including maternal (F0) function (Segment III-like):

- No effects on length of gestation or number of implantations (F0)
- No increase in stillbirths or decrease in live litter size
- Increased early postnatal deaths, dose-related, all doses (10, 20, and 40 mg/kg)
- Decreased pup birth weights at 20 and 40 mg/kg
- Decreased pup weights during lactation, dose-related, all doses
- Slightly increased latency to eye opening at 40 mg/kg
- Slightly increased latencies to develop several motor activities and reflexes (spontaneous activity of shoulder and pelvis (but not head), negative geotaxis, mid-air righting, cliff avoidance, clutching test, and auditory reflex.) especially at 20 and 40 mg/kg
- Learning and memory not tested.

Other parental effects:

- Slightly decreased maternal weight gain during last week of pregnancy and first 2 weeks post-parturition at 40 mg/kg
- Decreased food consumption, dose-related (all doses), throughout dosing period

Study no.: 651; accession no. (b) (4)-11852.
Volume #, and page #: volume 56, pages 84-206.
Conducting laboratory and location: Bristol-Myers co., Montpellier, France.
Date of study initiation: 3/17/86 (first day of mating).
GLP compliance: yes, see page 88.
QA reports: yes, see page 137.
Drug, lot #, and % purity: BMY 13805-1, lot no. 13, batch no. E84D079; purity not specified, but assayed at 99.5 and 100.3% by HPLC according to Certificates of analysis from 5/2/84 and 5/8/85 and Control Laboratory Report dated 1/21/87 (assay dated 2/5/87).
Formulation/vehicle: dissolved in deionized water (maximum concentration of 40 mg/10 ml = 4 mg/ml).

Methods:

Species/strain: male and female Sprague-Dawley rats (CD, (b) (4)); virgin females were mated with male rats (1 male and 2 females per cage); day of mating determined by appearance of copulatory plug and/or sperm in vaginal smear.
Doses employed: 0, 10, 20, 40 mg/kg/d.
Route of administration: oral gavage (10 ml/kg).
Study design: F0 pregnant female rats were dosed on days 15 of pregnancy through day 21 after delivery, when pups were weaned and group-caged by litter. F1 were not directly

exposed to drug, however, may have been exposed *in utero* or during lactation. F1 were tested for 1) physical development (2/sex/litter): body weight increase (at postnatal days 1, 4, 7, 14, 21), pinna detachment (day 3-6), hair growth (days 5-8), incisor eruption (days 7-15), eye opening (days 12-18), testes descent (days 20-27), vaginal opening (days 30 on); 2) motor and reflex development (another 2/sex/litter): spontaneous activity (days 3, 7, 11, 15), negative geotaxis (days 6, 8, 10, 12, 14), pivoting (day 3, 5, 7, 9, 11, 13), mid air righting reflex (days 10, 12, 14, 16, 18), cliff avoidance (days 3, 5, 7, 9, 11, 13), clutching test (days 8, 10, 12, 14), auditory reflex (days 10, 12, 14, 16, 18), olfactive discrimination (days 9, 10, 11, 12)
Number/sex/group: 20 pregnant (F_0) females/group.
Parameters and endpoints evaluated: F0: clinical signs (daily), body weight and food consumption (weekly), gestation duration, and necropsy (at day 21, number of implantations and resorptions counted). F1: number, weight and crown-rump length, sex, and external abnormalities (at birth), tests of physical development and motor and reflex development (during postnatal period, see specifics under "study design," above).

Results:
In-life observations, F0 dams:

Mortality: none.

Clinical signs: no abnormal findings; no sign of drug-related toxicity.

Body weight: HD dams tended to gain slightly less weight (only 14%, on average) during the last week of pregnancy (i.e., during dosing) than control and LD and MD dams (18-19%, on average for these groups). HD dams also tended to gain less weight during the first 2 weeks after parturition (~3% versus 8% in controls). All groups loss weight (~4%) at day 21 (vs day 14), presumably due to the stress of weaning.

Food consumption: Dosed dams ate less food than controls throughout the dosing period. HD dams ate 10% less during the last week of gestation and ~20% less during each of the 3 weeks after parturition; MD dams ate 8% less during the last week of gestation and ~12% less during each of the 3 weeks after parturition; LD dams ate 7% less during the last week of gestation and ~9% less during each of the 3 weeks after parturition.

Toxicokinetics: not examined; however, drug solutions (prepared on 4/29/86) were apparently analyzed on 5/14/86 and were within 1.5% of the nominal concentrations.

Mating and fertility: dosing inadequate for some (e.g., pre-implantation, early post-implantation) measurements, but no remarkable findings (see table below).

Table 54. Maternal variables.

PARAMETER	DOSE, mg/kg/d (gestational D15-LACTATION)			
	0	10	20	40
Pregnant Females at termination	**20**	**20**	**20**	**20**
Mean gestation duration, days	**21.6**	**21.9**	**22.0**	**22.1**
Pregnant females with live fetuses	**20**	**20**	**20**	**20**
Pregnant females with dead or aborted fetuses	0	0	0	**0**
Mean corpora lutea	14.6	15.8	17.5	**15.8**
Mean implantation sites	12.2	13.8	15.8	**12.0**
Mean resorptions	**0.90**	**1.40**	**1.00**	**1.25**
Mean live fetuses	**11.3**	**12.3**	**13.8**	**10.7**
Live feutses/implantation sites	**93%**	**89%**	**87%**	**89%**
Mean dead fetuses	**0**	**0**	**0**	**0**

Terminal and necroscopic evaluations (F0, day 21 after parturition): Apparently only macroscopic examination of (unspecified) organs was performed and did not display any abnormal findings correlated to treatment. Results of uterine exams were used to determine number of corpora lutea, etc. (see findings in table above).

In-life observations, F1 offspring:

Post-natal death of F1 offspring: Although there were no drug-related differences in litter sizes at birth (see table, above), many more drug-treated than control pups were found dead and/or cannibalized, especially during the first 4 days after birth (see table, below). The Sponsor's analysis was similar, finding decreased viability index on day 3 for HD pups.

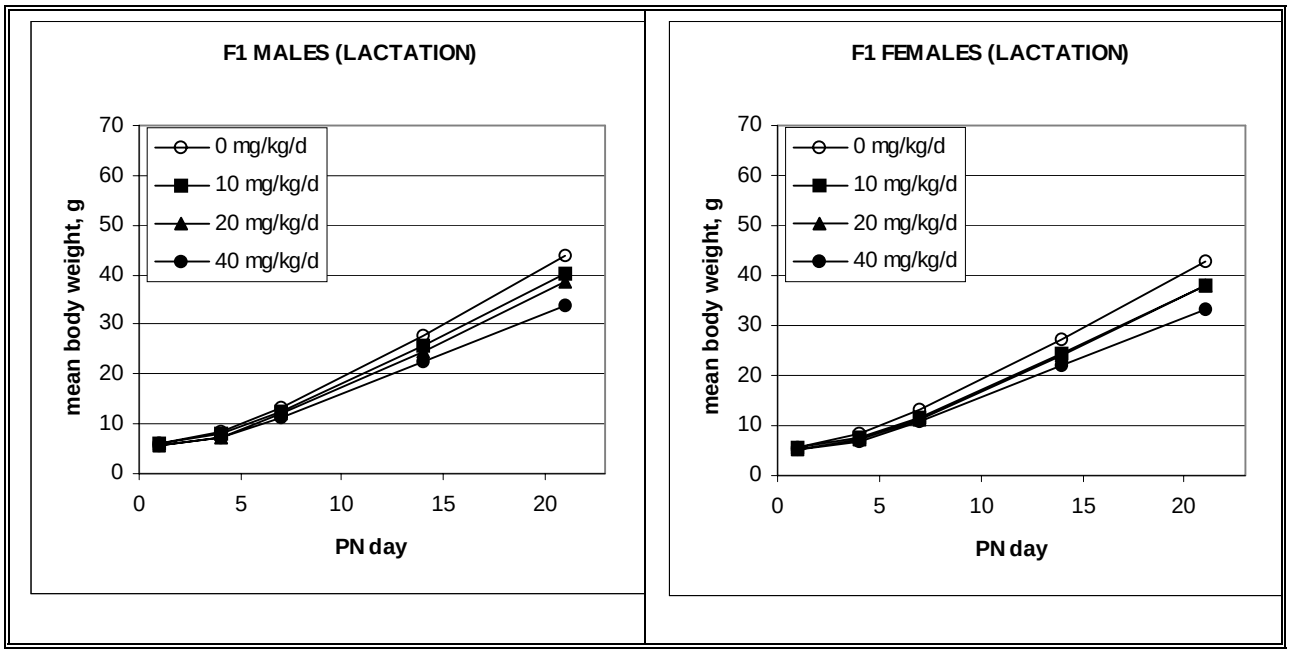
Body weights of F1 offspring: Birth weights for MD and HD groups were decreased ~10% compared with controls; by post-natal day 4, all drug-treated groups weighed less than controls and this persisted until at least day 16 (see table and Figure 23, below). It should be noted that there was no cross-fostering in this study, so post-natal effects on the pups' survival and body weights could stem from alterations in maternal and/or pup parameters (e.g., physiology and/or behavior).

Table 55. Geprione decreases pup size and survival in a Segment III study of 0, 10, 20, and 40 mg/kg oral (gavage) doses to rats.

PARAMETER	DOSE, mg/kg/d (gestational D15-LACTATION)			
	0	10	20	40
Total live litters	20	20	20	20
Mean birth weight, g (vs control)	5.84	5.66 (↓4%)	5.38* (↓9%)	5.31* (↓10%)
Mean birth length, mm	43.4	42.3	41.9	41.4
Total live births	226	246	276	214
Dead &/or cannibalized, days 1-4 (% of live births)	4 (1.8%)	17 (6.9%)	17 (6.2%)	39 (18%)
Dead &/or cannibalized, days 1-16 (% of live births)	5 (2.2%)	17 (6.9%)	23 (8.3%)	40 (19%)
Ratio of males:females	1.07	1.24	0.84	1.08
Mean day 4 weight, g (vs control)	8.57	7.99* (↓7%)	7.42* (↓13%)	7.05* (↓18%)
Mean day 21 weight, g (vs control)	43.8	40.3* (↓8%)	38.7* (↓12%)	34.0* (↓22%)

*: p<0.05, Dunnett's test.

Figure 23. Gepirone decreases F1-offspring body weights during lactation in a Segment III study in rats.



Physical development (F1): In general there were no drug-related changes in achievement of developmental landmarks (see Table 56, below). The slightly earlier appearance of hair growth at LD and MD, though statistically significant, seems of doubtful relevance. The slightly delayed eye opening at HD could reflect slightly delayed development, correlating with decreased birth weights and decreased weights during the post-natal period; however, MD pups showed similarly decreased weights, without an effect on eye-opening time.

Table 56. The Sponsor's table showing physical development of pups (F1). Values represent the day when 50% of each dose-group tested positive for each trait. Dose groups are controls (1), 10 mg/kg/d (2), 20 mg/kg/d (3), and 40 mg/kg/d (4); dosing of dams from gestational day 15 through post-natal day 21 (i.e., through lactation).

Group n°	1	2	3	4
Pinna detachment	3.4	3.4	3.5	3.7
Hair growth	6.1	5.5**	5.5**	6.2
Incisor eruption				
- inferior	11.2	11.4	11.4	11.1
- superior	10.6	11.0	10.9	10.8
Eye opening	14.5	14.6	14.3	15.1**
Descent of testes	21.7	20.7	21.1	21.9
Vaginal opening	33.5	33.9	33.3	34.0

Statistical evaluation :

* = $p < 0.05$

** = $p < 0.01$

*** = $p < 0.001$

Motor and reflex development in F1 offspring (see Table 57, below): Delayed development in F1 offspring of dams treated with MD and/or HD was evident in the significantly delayed development of several behaviors, viz., spontaneous activity of shoulder and pelvis (but not head), negative geotaxis, mid-air righting, cliff avoidance, clutching test, and auditory reflex. Mid-air righting and cliff avoidance were also delayed in F1 offspring of LD dams.

Table 57. The Sponsor's table showing motor and reflex development of pups (F1). Values represent the day when 50% of each dose-group tested positive for each trait. Dams were dosed from gestational day 15 through post-natal day 21 (i.e., through lactation).

Group n°	1	2	3	4
BMY-13805-1 mg/kg/day	0	10	20	40
Spontaneous activity				
- Head	6.4	7.2	7.2	7.1
- Shoulder	8.4	8.8	9.9**	8.5
- Pelvis	9.7	10.3	10.9*	10.3
Negative geotaxis	7.7	8.3	8.6*	8.7
Pivoting	5.6	5.6	5.9	5.1
Mid air righting	14.2	15.0*	14.8	15.0*
Cliff avoidance	5.7	7.0*	6.4	6.5
Clutching test	7.6	7.6	7.7	8.4*
Auditory reflex	13.5	13.7	13.6	13.9*
Olfactive discrimination	9.1	8.6	9.1	9.0
Statistical evaluation				
* = p<0.05				
** = p<0.01				
*** = p<0.001				

Summary of individual study findings: See key findings at beginning of this study.

Reproductive and developmental toxicology summary and conclusions: See overall summary in Detailed Conclusions and Recommendations section VIII.

Labeling recommendations:

(b) (4)

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VIII. SPECIAL TOXICOLOGY STUDIES:

None submitted.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Summary and Conclusions:

Pharmacology: Gepirone binds selectively with moderate affinity to serotonergic 5-HT_{1A} receptors *in vitro,* with some indication of possible activity at some D2-type dopamine receptors. The Sponsor claims that gepirone is an agonist at presynaptic receptors and a partial agonist at postsynaptic receptors, however, the evidence for partial vs full agonism is not compelling. The actual mechanism of action of gepirone as an antidepressant, as for all antidepressants, is unknown. As for other currently approved antidepressants, the actual mechanism of action is probably through (as yet unidentified/unexplained) compensatory mechanisms initiated by the direct effect(s) of the drugs. Gepirone will be the first drug in it's pharmacological and chemical/structural class to be approved as an antidepressant, however, buspirone, which is nearly identical in structure and pharmacology, has already been approved as an anxiolytic drug.

Although geprione appears fairly selective for 5-HT_{1A} receptors, 1-PP (a major metabolite in humans) has antagonist activity at $alpha_2$ adrenergic (auto) receptors, increasing norepinephrine release and activity. The other major human metabolite, 3'-OH-gepirone, has affinity for 5-HT_{1A} receptors similar to that of gepirone, however, its selectivity for this receptor subtype was not well characterized (only a limited number of receptors was investigated; notably absent were other 5-HT_1-subtypes). It would also be of interest to know whether gepirone or its metabolites have antagonist activity at monoamine oxidase (MAO) or biogenic amine transporters, such at serotonin reuptake transporter, like other classes of antidepressants. I could not find compelling evidence in the current submission regarding these issues: the evidence that gepirone did not inhibit these protein targets was weak and there was some evidence that a major human metabolite (1-PP) blocked the serotonin transporter.

The Sponsor seems to feel that gepirone has no meaningful affinity for dopamine receptors and contrasts this favorably with buspirone's apparently higher affinity, but this is not clear from the binding data referenced in this submission. Gepirone does seem to have low or no affinity at D_1 dopamine receptors (displacement of SCH 23390) and D_2 dopamine receptors (displacement of spiperone) from rat brain or striatal membranes. However, in the studies using cloned receptors of the D_2 class (i., D_2, D_3, and D_4), there is some discrepancy. Gepirone had moderate affinity at cloned rat D_2 receptors (Chio, 1990) with a Ki of 58 nM (vs U-86170). In human clones of D_2-type receptors, gepirone showed weak if any affinity at D_{2L} and D_{2S} forms, but moderate affinity at 2 other D_2-type receptors (namely, D_3 and D_4).

It should also be reiterated that compensatory changes at other points in the serotonergic system (e.g., down-regulation of 5-HT_2 receptors noted in rats) or in other systems, initiated by direct activity at 5-HT_{1A} receptors, may underlie pharmacological and toxicological responses after repeated administration of gepirone.

Safety pharmacology: Although safety pharmacology studies showed some effects of gepirone on cardiac and renal function, these would be monitorable in humans. Gepirone appears to have some proconvulsant potential, based upon potentiation of seizures induced by strychnine and

picrotoxin in rats. Gepirone does not appear to have abuse potential, based upon the animal studies and literature references submitted with this application.

ADME: Gepirone is heavily metabolized in humans and animals. In humans, 2 major metabolites of gepirone have been identified: 1-PP (which is also a metabolite of buspirone, currently approved and marketed as an anxiolytic) and 3'-OH-gepirone. Both of these metabolites have been identified and found to be present in substantial amounts (many times the levels of gepirone) in plasma of the 2 major species used in the preclinical studies, namely rats and dogs, following gepirone administration. In vitro studies have shown that these metabolites can be made by liver microsomes from mice, as well as rats, dogs, and humans.

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

In the 1-year dietary study in rats (4, 12→16, and 36→48 mg/kg/day; MD and HD increased as indicated at week 19), doses were limited by decreases in body weights. Toxicities were limited to slightly increased incidence of histiocytosis in lungs at the high dose, distended bladders seen at necropsy in high dose males, and some changes in organ weights (increased weights of prostate and ovaries, decreased weights of uteruses) that might indicate "endocrine effects," a not unexpected effect of serotonergic compounds in rats.

In the 1-year study in dogs (4, 8, and 16 mg/kg/day orally, in a (b) (4) capsule), decreases in body weights (and food consumption at the HD) limited doses. Aside from clinical signs (e.g., hypoactivity, salivation, increased respiratory rate), the only drug-related finding was decreased erythrocyte sedimentation rate seen throughout the study in high dose males only.

Mutagenicity: Gepirone was not genotoxic in 2 out of 3 tests that form the Standard Battery, according to the current ICH Guidance (1997). It was no mutagenic in the *in vitro* bacterial reverse mutation assay (Ames test) and was not clastogenic in an *in vivo* chromosomal aberration test of rat bone marrow cells, but was not adequately tested in an *in vitro* chromosomal aberration test. Additionally, gepirone was not mutagenic in an *in vitro* mammalian forward gene mutation assay in CHO/HGPRT cells or in an *in vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes.

Carcinogenicity: Although the carcinogenicity studies were performed prior to the establishment of the CAC within the Agency, the dietary doses used in the studies were based upon decreases in body weights or weight gains from dietary studies of at least 13-week duration and are in agreement with the currently accepted standards for dose-selection. Palatability did not appear to be an issue; food consumption was not affected. This reviewer agreed with the Sponsor that there were no gepirone-related tumors evident in either rats or mice up to maximum tolerated doses (MTD); concurrence by the Executive-CAC was given on January 8, 2002.

Reproductive toxicity: Gepirone was adequately tested for reproductive toxicity in: 1) Combination studies (i.e., Segments I and III) in rats, with oral gavage dosing of F0 males and females before and during mating, and females through pregnancy and lactation; following F1

offspring to sexual maturity; 2) traditional Segment II studies, in rats and rabbits, with oral gavage dosing of pregnant females during the period of organogenesis; and 3) a traditional Segment III study in rats, with oral gavage dosing of pregnant females from late pregnancy through lactation; F1 offspring were only followed through lactation, not to sexual maturity in this study.

Gepirone showed some adverse action on fertility in rats. When gepirone was administered orally to male and female rats prior to and throughout mating at daily doses of 5, 27, 64, and 150 mg/kg, the latency to mating was increased at doses of 64 mg/kg and above. Fetal weight was decreased at 27 mg/kg and above and fetal length was decreased at 64 mg/kg and above. It is interesting to speculate that the apparent decrease in mating behavior seen with gepirone in rats may relate to the decreased libido seen in humans treated with serotonin reuptake inhibitors.

Gepirone also had adverse effects on embryo/fetal and postnatal development in rats and rabbits. In embryo/fetal development studies, oral administration of gepirone to pregnant rats (75, 150, and 300 mg/kg/day) or pregnant rabbits (50, 100, and 200 mg/kg/day) during the period of organogenesis resulted in decreased embryo/fetal body weights and lengths, with accompanying skeletal variations, at the mid and high doses. No teratogenic effects were seen in these studies.

In a study of prenatal and postnatal development, when pregnant rats were treated with gepirone (10, 20, and 40 mg/kg/day) from late gestation through weaning, decreased birth weights were seen at 20 mg/kg and above. Increased offspring mortality during the first 4 days after birth and persistent offspring growth retardation were observed at all doses. The no-effect dose for fetal effects was not determined in this study.

When gepirone was administered orally to male and female rats prior to and throughout mating, gestation and lactation at doses of 5, 27, 64, and 150 mg/kg/day, increased still births were seen at 64 mg/kg and above. Early postnatal mortality was increased at 150 mg/kg. Pup weights were decreased at birth, throughout lactation and weaning, and until at least 14 weeks of age, with delays of some developmental landmarks, at 64 mg/kg and above. The no-effect dose for all these effects on offspring was 27 mg/kg. It is also interesting that decrements in sexual performance were also seen for the F1 offspring, especially increased early postnatal losses of F2 pups. The number of F1 pregnancies that were examined was small, but the effect was seen in 2 studies: at 150 mg/kg dose to F0 rats in one study and at 64 mg/kg in a second study.

General Toxicology Issues:

The *in vitro* chromosomal aberration test (1 out of 3 tests that form the standard battery for testing mutagenicity, according to the 1997 ICH Guidance, S2B Genotoxicity) was not adequate by current standards.

In preclinical reproductive toxicity studies, there were consistent effects of geprione on fetal and maternal variables, especially on fetal sizes (decreased) and early postnatal survival of pups. These effects were seen at doses of gepirone that decreased maternal body weights or weight gains enough to indicate the adequacy of dosing for the validity of the studies. However, the decrements in maternal weights were not of a magnitude that would compromise the findings of these studies.

Recommendations: The *in vitro* chromosomal aberration test, part of the standard test battery according to the current ICH Guidance for Industry, S2B Genotoxicity, 1997, was inadequate and should be repeated. In the submitted study, gepirone was negative for 5-hr treatment, with and without metabolic activation. However, the study was not valid, because this negative finding (without activation) should have been followed up with a study using continuous treatment with gepirone (without activation) for ~24 hr (1.5 cell doubling times), in accordance with the current ICH Guidance.

Because gepirone was not mutagenic or clastogenic in the other 2 tests from the Standard Battery, as specified in the current ICH Guidance (1997) (and not mutagenic in 2 other *in vitro* tests) and was not carcinogenic in rat or mouse 2-year bioassays, a Phase IV commitment for this study would be acceptable.

Because of the effects of gepirone in the reproductive toxicity studies (e.g., decreased fetal body weights and decreased pup survival), gepirone should be classified as Category C, and the results of the studies described in labeling (see proposed labeling section).

Labeling with basis for findings:

CLINICAL PHARMACOLOGY

(b) (4)

[Explanatory notes:

- The Sponsor asserts in labeling that “ (b) (4)

(b) (4)

”

This detail regarding the exact mechanism of action of gepirone is speculative.

- The Sponsor asserts in labeling that gepirone has (b) (4)

”

The evidence from the studies supporting these claims is weak and does not take into consideration the binding of major metabolites of gepirone. 3’-OH-geprione has moderate affinity for 5-HT_{1A} receptors similar to that of geprione. 1-PP binds with moderate affinity to alpha2 adrenergic receptors. None of the 3 seem to have significant affinity for muscarinic cholinergic receptors; and the human side-effect profile does not suggest muscarinic activity.]

(b) (4)

(b) (4)

[The Sponsor proposed "(b) (4) " This wording is stronger than the data warrants.]

X. APPENDIX/ATTACHMENTS:

Addenda to review:

Minutes of the January 8, 2002, meeting of the Executive-CAC meeting.

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/s/

Joe Contrera
1/15/02 09:27:33 AM

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/s/

Linda Fossom
3/8/02 01:40:31 PM
PHARMACOLOGIST

Barry Rosloff
3/8/02 02:28:15 PM
PHARMACOLOGIST
Agree with all recommedations except I think the labeling should not include (b) (4) .