

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:NDA 20937

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Division of Medical Imaging and Radiopharmaceutical Drug Products
(HFD-160)

NDA 20-937

Drug product: Optimark

Sponsor: Mallinckrodt, Inc.
St. Louis, MO

Documents covered in this review:

<u>Serial #</u>	<u>Document Date</u>	<u>Document Type</u>
M	10/30/97	presubmission
N 000	3/2/98	Original
	3/23/98	BZ
	5/22/98	BP
	5/29/98	BP

Completion Date: 12/1/98

Information to sponsor: yes (X) no ()
Information to review team: yes () no (X)

Related IND: IND

Disclaimer: Some of the information in this review has been copied from the Sponsor's NDA submission.

Quotations from the Sponsor's submission are in *italics*; other copied information is appropriately identified.

Drug Class: Magnetic resonance imaging (MRI) contrast enhancing agent

Proposed indication (quoted from the proposed package insert):

Optimark Injection is indicated for use with magnetic resonance imaging (MRI) in adults to provide contrast enhancement in those intracranial lesions with abnormal vascularity or those thought to cause abnormalities in the blood-brain barrier. Optimark injection has been shown to facilitate visualization of intracranial lesions including but not limited to tumors.

Optimark Injection is also indicated for use with MRI in adults to provide contrast enhancement and facilitate visualization of lesions of the spine and associated tissues.

Optimark Injection is also indicated for use with MRI in adults to provide contrast enhancement and facilitate visualization of lesions in the liver.

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List Of Submitted Studies (Reviewed studies are bolded).

	NDA Summary Page	Full Report Page
Pharmaco-/Toxicokinetics Studies		
1.-20. Pharmaco- and toxicokinetic studies were reviewed separately in Pharm/Tox Review #2 for NDA 20-237. The summary of those studies is included in this review.		
Pharmacology Studies		
21. An Investigation of the Properties of OptiMARK™ for MRI Enhancement of Cerebral Metastatic Disease Part A: Illustrative case reports, dose comparisons, contrast enhancement values, macrohistopathology Part B: Tumor permeability determination Part C: Blinded neuroradiological interpretation, microhistopathology	14.126	17.157
22. Direct Comparison of Renal MR Contrast Enhancement using MP-1177/10 Injection and Magnevist® (Study 1101/05/92/036-E)	14.230	32.001
23. Molar Relaxivity Rates of MP-1177/10 and Magnevist® in Water and BSA Solution (Study 1101/05/92/078-E)	14.231	32.020
Safety Pharmacology Studies		
24. Hemodynamic Effects of MP-1177/10 Injection, Injected at a Dose Rate of 1.0 mL/kg/min in Anesthetized Dogs (Study 1101/05/92/061)	14.126	17.109
25. Hemodynamic Effects of MP-1177/10 with 10% Excess Ligand in Anesthetized Dogs after Intravenous Administration (Study 1101/05/92/028)	14.125	17.055
26. A Comparison of the Hemodynamic Effects of MP-1177/10 Injection and Magnevist®		

	in Anesthetized Dogs (Study 1101/05/93/015-E)	14.227	31.257
27.	Effect of MP-1177/10 and Magnevist® on the Isolated Rat Aorta (Study 1101/05/93/029-E)	14.228	31.287
28.	General Pharmacological Study of MP-1177	14.121	17.019b

Single dose Toxicology Studies

Pivotal studies (bolded) are reviewed individually. All other single dose studies are summarized in tables in the single dose toxicology section.

29.	MP-1177/10T: Acute Intravenous Toxicity Study in the Mouse (Study 1101/05/91/027)	14.129	17.230
30.	Acute Intravenous Toxicity of MP-1177/10 in Mice (Study 1101/05/92/003)	14.131	17.282
31.	Part A: Acute Intravenous Toxicity of MP-1177 in Mice (Study 1101/05/92/031)	14.201	28.134
32.	Acute Intravenous Toxicity of MP-1177/10 Injection to Determine the Maximal Non-Lethal Dose in ICR Mice (Study 1101/05/94/008)	14.131	17.311
33.	Comparison of the Acute Intravenous Toxicity of MP-1177/10 Injection to that of Magnevist® in Mice (Study 1101/05/93/021)	14.232	32.032
34.	Comparison of the Acute Intravenous Toxicity of MP-1177/10 Injection to that of Magnevist® in Mice (Study 1270/05/93/038)	14.233	32.068
35.	The Effect of MP-1196 and Calcium on the Acute Toxicity of MP-1177 in Mice (Study 1101/05/90/024-E)	14.206	28.230
36.	Acute Intravenous Toxicity of Ca ²⁺ MP-1196 in Mice (Study 1101/05/92/046)	14.210	29.008
37.	Part B: Acute Intravenous Toxicity of 2-Methoxyethylamine in Mice (Study 1101/05/92/031)	14.225	31.215

38.	Comparison of the LD ₅₀ of 4 Different Lots of MP-1177/10 Injection in Mice (Memo-to-File 1101/05/93/018-E)	14.193	28.006
39.	Comparison Study of the Acute Intravenous Toxicity of Three Lots of MP-1177/10 Injection on ICR Mice (Study 1101/05/93/050)	14.195	28.026
40.	A 2-Week Single Dose Intravenous Toxicity Study of MP-1177/10 in the Albino Rat (Study 1101/05/94/028)	14.146	21.182
41.	Preliminary Acute Histotoxicology Study of the Effect of MP-1177/10 Injection on the Kidneys and Testes of the Rat (Memo-to-File 1101/03/95/014-E)	14.144	21.158
42.	Acute Intracisternal Toxicity of MP-1177/10 in Rats (Study 1101/05/92/004)	14.134	18.070
43.	A Toxicity Study of MP-1196 by Single Intravenous Administration in Rats	14.211	29.033
44.	An Acute Toxicity Study of MP-1177/10T in the Dog via Intravenous Injection (Study 1101/05/91/032)	14.134	18.001
45.	A Toxicity Study of MP-1196 by Single Intravenous Administration in Beagle Dogs	14.219	31.001
46.	Comparison Study of the Acute Intravenous Toxicity of MP-1177/10 Injection in a Plastic Syringe Compared With a Standard Lot of MP-1177/10 Injection in Glass Vials in ICR Mice (Study 1101/05/94/016-E) (To be reviewed in NDA 20-976)	14.197	28.067

Multiple Dose Toxicology Studies

47.	MP-1177/10T: Toxicity Study by Intravenous Administration to CD Rats for 4-Weeks Followed by 4-Week and 8-Week Reversibility Periods (Study 1101/05/91/029)	14.136	18.114
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48.	A Subchronic (4-Week) Toxicity Study of MP-1177/10T in the Dog via Intravenous Injection with an 8-Week Recovery Period (Study 1101/05/91/033)	14.141	20.001
49.	A 4-Week Toxicity Study of MP-1196 by Repeated Intravenous Administration in the Rat Followed by a 4-Week Recovery Period	14.212	29.106
50.	A 4-Week Toxicity Study of MP-1196 by Repeated Intravenous Administration in Rats	14.217	30.180
51.	A 2-Week Toxicity Study of MP-1196 by Repeated Intravenous Administration in Beagle Dogs (Preliminary Study)	14.220	31.034
52.	A 4-Week Toxicity Study of MP-1196 by Repeated Intravenous Administration in Beagle Dogs Followed by a 4-Week Recovery Period	14.222	31.069
53.	Five-Day Repeat Dose-Toxicity of MP-1177 and Gadolinium Citrate in Rats (Study 1101/05/91/011-E)	14.207	28.262
Carcinogenicity Statement		14.148	N/A
Reproductive Toxicology			
54.	A Study of the Effect of MP-1177/10 on Fertility and General Reproductive Performance on the Rat (Study 1101/05/92/017)	14.152	22.007
55.	A Study of the Effect of MP-1177/10 on the Pregnancy of the Rat (Study 1101/05/92/022)	14.156	23.001
56.	A Pilot Study of the Effect of MP-1177/10 on the Female Rabbit (Study 1101/05/92/038-E)	14.158	23.216
57.	A Preliminary Study of the Effect of MP-1177/10 on Pregnancy of the Rabbit		

(Study 1101/05/92/023-E)

14.159 23.235

58. A Study of the Effect of MP-1177/10 on Pregnancy of the Rabbit (Study 1101/05/92/024)

14.161 23.287

59. A Preliminary Study of the Effect of MP-1177/10 on the Pregnant Rat and Offspring During the Peri- and Post Natal Period (to Day 8 Postpartum) (Study 1101/05/92/016-E)

14.162 24.001

60. A Study of the Effect of MP-1177/10 on the Pregnant Rat and Offspring During the Peri- and Post Natal Period (Study 1101/05/92/025)

14.164 24.051

Genotoxicology

61. Salmonella / Mammalian Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and Escherichia Coli WP2 UVRA Reverse Mutation Assay with a Confirmatory Assay (Study 1101/05/92/012)

14.167 24.253

62. L5178Y TK +/- Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay (Study 1101/05/92/013)

14.167 24.324

63. Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells (Study 1101/05/92/014)

14.168 24.366

64. Micronucleus Cytogenetic Assay in Mice (Study 1101/05/92/015)

14.169 24.424

Special Toxicology

65. Venous Irritation of MP-1177/10 Injection in Rabbits (Study 1101/05/93/011)

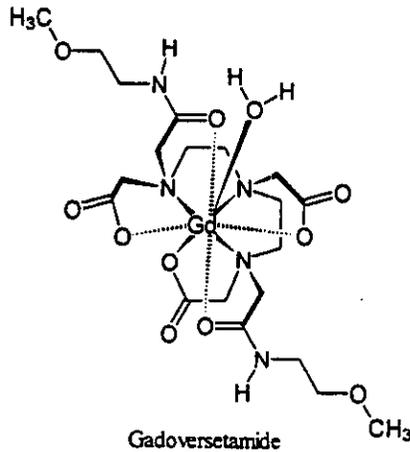
14.149 21.238

66. Comparative Study of the Venous Irritation of MP-1177/10 Injection and Magnevist® in

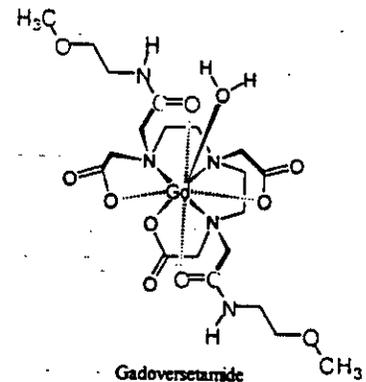
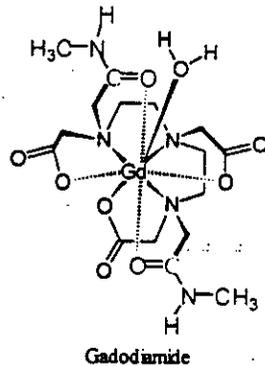
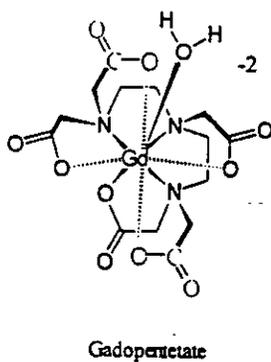
	Rabbits (Study 1101/05/93/016)	14.235	32.131
67.	Intramuscular and Subcutaneous Irritation of MP-1177/10 in Rabbits (Study 1101/05/92/029)	14.150	21.309
68.	In vitro Human Blood Compatibility of MP-1177/10 (Study 1101/05/92/019)	14.173	25.122
69.	Comparative Blood and Plasma Compatibility of MP-1177/10 Injection and Magnevist® (Study 1101/05/93/022)	14.238	32.306
70.	Antigenicity Test of MP-1177	14.202	28.170
71.	Primary Screening of a Test Article for Antagonism to Histamine Using the Isolated Guinea Pig Trachea (Pilot Study) (Study 1101/05/93/027-E)	14.229	31.305
72.	Primary Screening of MP-1177/10 and Magnevist® for Effects Upon Histamine or Acetylcholine Induced Contraction Using the Isolated Guinea Pig Trachea (Study 1101/05/93/028-E)	14.229	31.318
73.	Neurotoxicity of Extracellular MRI Agents (Study 1101/05/93/031)	14.236	32.190

Relevant Chemistry Information

- Chemical name: Gadoversetamide injection
- CAS number:
- Chemical structure of drug substance:



The structures of gadopentetate (Magnevist) and gadodiamide (Omniscan) are presented for comparison to gadoversetamide (Optimark). Gadopentetate is simply Gd-DTPA. The 2 organic acid moieties of Gd-DTPA are substituted with methylamide groups in gadodiamide, and with methoxyethylamide groups in gadoversetamide, as shown:



- Molecular weight gadoversetamide: 662 g/mole

Clinical Formulation

Section 4A3:2.1 Statement of Quantitative Composition

The following is a list of ingredients and the target quantity, release limit, and quantity per 950 L formulated OptiMARK batch, which is the largest commercial batch size.

<u>Ingredient</u>	<u>Target</u> <u>Quantity</u>	<u>Release</u> <u>Limit</u>	<u>Quantity per</u>
↓ <u>Gadoversetamide*</u>			
↓ <u>Versetamide*</u>			
↓ <u>Calcium hydroxide, USP</u>			
↓ <u>Calcium chloride dihydrate,</u> <u>USP</u>			
↓ <u>Sodium hydroxide, NF</u>			
↓ <u>Hydrochloric acid, NF</u>			
↓ <u>Water for Injection, USP</u>			

*correct for moisture content

**input quantities of calcium hydroxide and calcium chloride dihydrate verified by assay for total Ca⁺⁺.

- a. Route of administration: intravenous
- b. Injection rate: bolus injection
- c. Dose schedule: single administration
- d. Clinical Dose: 0.1 mmole/kg (0.2 ml/kg)

Proposed Clinical Doses of Optimark Components		
Component	Molar Dose (mmole/kg)	Mass Dose (mg/kg)
gadoversetamide	0.1	66.18
[elemental gadolinium]	0.1	15.30
versetamide	0.01	5.08
calcium hydroxide	0.01	0.74
calcium chloride	0.001	0.15
sodium hydroxide	dose depends on amount needed for pH adjustment	
hydrochloric acid	dose depends on amount needed for pH adjustment	
water for injection	<0.2 ml/kg	

**APPEARS THIS WAY
ON ORIGINAL**

Animal Formulations:

Several formulations were administered in animal studies:

- 1) MP-1177/10T This is an old formulation of Optimark which contained TRIS buffer
- 2) MP-1177/10 Optimark
- 3) Gd-153 MP-1177/10 radiolabeled formulation of Optimark for pharmacological and toxicokinetic studies.
- 4) MP-1196 versetamide (note that MP-1196 is not calcium versetamide)
- 5) 2-MEA 2-methoxyamine, a impurity
- 6) gadolinium citrate

Comparison of 2 formulations of Optimark		
	MP-1177/10T (early)	MP-1177/10 (final)
Gadoversetamide (MP-1177)	mg/mL	mg/mL
	mg/mL	mg/mL
TRIS*	mg/ml mM)	
CaCl ₂	mg/mL	mg/mL
Total (MP-1196)	mg/mL	mg/mL
2-MEA	** mg/mL	mg/mL
MP-450	** mg/mL	mg/mL
MP-1832	** mg/mL	mg/mL
MP-2077	** mg/mL	mg/mL
Heavy Metals	mg/mL	mg/mL
*TRIS is 2-amino-2-(hydroxymethyl)-1,3-propanediol also known as tromethamine **impurity profiles were not defined in MP-1177/10T, the early formulation		

Formulations for animal studies

STUDY #	FORMULATION	LOT #	PASS/FAIL Release test
ADME			
1. 1101/05/92/008	MP-1177/10	B1658p57	Pass
		B1658p35B	Fail
2. 1101/05/92/054-E	MP-1177/10	B1749p30	Pass
3. 1101/05/92/087	MP-1177/10	B1749p30	Pass
4.	MP-1177/10	CRM3580	Pass
	MP-1177/10	CRM3571	Pass
5. 1101/05/92/041-E	MP-1177/10	B1658p57	Pass
6. 1101/05/92/007	MP-1177/10	B1658p35-B	Fail
		B1658p35-C	Pass
7.	MP-1177/10	CRM3580	Pass
	MP-1177/10	CRM3571	Pass
8. 1101/05/92/042	[153Gd] MP-1177/10	B1658p35B	Fail
9. 1101/05/92/075	MP-1177/10	S92162	Pass
10.	MP-1177/10	CRM3580	Pass
	MP-1177/10	CRM3571	Pass
11. 1101/05/92/051	MP-1177/10	S92162	Pass
12. 1101/00/94/002	MP-1177/10	S92162	Pass
13. 1101/05/94/018	MP-1177/10	B2072p28	Fail
14. 1101/05/94/026-E	MP-1177/10	S91426	Pass
	MP-1177/10	S91427	Pass
15. 1101/05/92/053	MP-1177/10	B1749p20	Fail
16. 1101/00/92/022	MP-1177/10	E9205PR	Pass
17. 1101/00/92/023	MP-1177/10	S91144	Pass
18. 1101/00/92/020	MP-1177/10	E9205PR	Pass
19. 1101/00/92/021	MP-1177/10	S91144	Pass
20. 1101/05/93/032-E	MP-1177/10	E9205PR	Pass
PHARMACOLOGY			
21.	MP-1177/10	E9205PR	Pass
22. 1101/05/92/036-E	MP-1177/10	S91144	Pass
23. 1101/05/92/078-E	MP-1177/10	S92162	Pass
SAFETY			
PHARMACOLOGY			
24. 1101/05/92/028	MP-1177/10	B1658p57	Pass
25. 1101/05/92/061	MP-1177/10	S29120-C	Pass
26. 1101/05/93/015-E	MP-1177/10	E9205PR	Pass
27. 1101/05/93/029-E	MP-1177/10	E9205PR	Pass
28.		B1658p57	Pass
SINGLE DOSE			
29. 1101/05/91/027	MP-1177/10T	B1432p80	Pass
30. 1101/05/92/003	MP-1177/10	S91144	Pass
31. 1101/05/92/031A	MP-1177	B1658p69	Pass
32. 1101/05/94/008	MP-1177/10	S94110-A	Pass
33. 1101/05/93/021	MP-1177/10	E9205PR	Pass
34. 1270/05/93/038	MP-1177/10	C9307PR	Pass
35. 1101/05/90/024-E	MP-1177/10	Formula 1:B1432p34B	Pass
	MP-1177/10	Formula 1:B1432p34C	Pass

Summary of Single Dose Studies-Optimark-DOSE INFORMATION AND TERMINATION TIMES										
#	Report #	species/ strain	test subst	n/gr		route	doses (mmole/kg unless otherwise stated)	dose volumes (ml/kg)	Inj rate ml/min	Term times -days
				♂	♀					
29	1101/05/91/027	mouse	MP-1177/10T	5	5	iv	0,10,20,25,30	60,20,40,50,60	1	14
30	1101/05/92/003	mouse	MP-1177/10	5	5	iv	0,0,24,26,28,30	0,60,48,52,56,60	1	14
31	1101/05/92/031A	mouse	MP-1177	5	5	iv	0,8,10,12,14,16	28,16,20,24,28,32	1	14
32	1101/05/94/008	mouse	MP-1177/10	5	5	iv	0,12,14,16,18,20	40,24,28,32,36,40	1	14
33	1101/05/93/021	mouse	Optimark, Magnevist	5	5	iv	Opt 0, 7 to 24 Mag 0,4,5,6	Opt 60,14 to 48 Mag 60,8,10,12	1	14
34	1270/05/93/038	mouse	Optimark, Magnevist	5	5	iv	Opt 0, 16 to 28 Mag 0,2,4,6	Opt 60, 32-56 Mag 60, 4, 8, 12	1	14
35*	1101/05/90/024-E	mouse	MP-1177 plus MP-1196 and Ca	0	1-4	iv	MP-1177 6-36 MP-1196 1-5 % Ca 1-6 %	12-72	?	7
36	1101/05/92/046	mouse	MP-1196	5	5	iv	0, 0, 10 to 18	33, 37, 20 to 37	1	14
37	1101/05/92/031B	mouse	2-methoxyamine (an impurity)	5	5	iv	0,6,8,10,12	24,12,16,20,24	1	14
38	1101/05/93/018-E	mouse	MP-1177/10-4 lots incl lots from 30. and 33.	5	0-5	iv	0,20 to 30	60,40 to 60	1	4
39	1101/05/93/050	mouse	MP-1177/10-3 lots (test maufact change)	5	5	iv	0,16,20,24,28	60,32,40,48,56	1	14
40*	1101/05/94/028	rat	MP-1177/10	10	0	iv	0,5,5,15	30,1,10,30	1	1,14
41	1101/03/95/014-E	rat	MP-1177/10	2	0	iv	4,14,16, no controls	8,28,32	1	1,7
42*	1101/05/92/004	rat	MP-1177/10	5	5	i.c.	0,0,50-200 umole/kg	0,400,100-400 ul/kg	50ul/sec	14
43	Bozo B-2481	rat	MP-1196	5	5	iv	0,1,2,4	10,10,10,10	1	14
44*	1101/05/91/032	beagle dog	MP-1177/10T	2	2	iv	0,3,6,12	24,6,12,24	4-11	14
45	Bozo B-2483	beagle dog	MP-1196	1	1	iv	1,2,4, no controls	10,10,10	10	14
46	1101/05/94/016-E	mouse	MP-1177/10	to be reviewed in NDA 20-976						

*reviewed in detail in text

Summary of Single Dose Studies-Optimark-MEASURES OF TOXICITY													
(✓ indicates measurement made)													
#	Report #	species/ strain	test substance	mort	tox sign	bw	fc	cl ch	hem	urin	org wt	gr path	hist path
29	1101/05/91/027	mouse	MP-1177/10T	✓	✓	✓	-	-	-	-	-	✓	-
30	1101/05/92/003	mouse	MP-1177/10	✓	✓	✓	-	-	-	-	-	✓	-
31	1101/05/92/031A	mouse	MP-1177	✓	✓	✓	-	-	-	-	-	✓	-
32	1101/05/94/008	mouse	MP-1177/10	✓	✓	✓	-	-	-	-	-	✓	✓ if abnormal
33	1101/05/93/021	mouse	Optimark, Magnevist	✓	✓	✓	-	-	-	-	-	✓	-
34	1270/05/93/038	mouse	Optimark, Magnevist	✓	✓	✓	-	-	-	-	-	✓	✓
35*	1101/05/90/024-E	mouse	MP-1177 plus MP-1196 and Ca	✓	✓	✓	-	-	-	-	-	✓	-
36	1101/05/92/046	mouse	MP-1196	✓	✓	✓	-	-	-	-	-	✓	-
37	1101/05/92/031B	mouse	2-methoxyamine (an impurity)	✓	✓	-	-	-	-	-	-	✓	-
38	1101/05/93/018-E	mouse	MP-1177/10-4 lots (incl from 30. and 33.)	✓	✓	-	-	-	-	-	-	✓ 1 or 2 /gr	-
39	1101/05/93/050	mouse	MP-1177/10-3 lots (test maufact change)	✓	✓	✓	-	-	-	-	-	✓	-
40*	1101/05/94/028	rat	MP-1177/10	✓	✓	✓	-	-	-	-	✓	✓	✓ kidney testis epididym
41	1101/03/95/014-E	rat	MP-1177/10	✓	-	-	-	-	-	-	✓ kidn testis	-	✓ kidney testis epididym
42*	1101/05/92/004	rat	MP-1177/10	✓	✓	✓	-	-	-	-	✓ brain	✓ brain	-
43	Bozo B-2481	rat	MP-1196	✓	✓	✓	-	-	-	-	-	✓	-
44*	1101/05/91/032	beagle dog	MP-1177/10T	✓	✓	✓	-	✓	✓	-	✓	✓	-
45	Bozo B-2483	beagle dog	MP-1196	✓	✓	✓	✓	-	-	-	✓	✓	✓ 7 orgs/tissu
46	1101/05/94/016-E	mouse	MP-1177/10	to be reviewed in NDA 20-976									

*reviewed in detail in text

Summary of Single Dose Studies-Optimark-RESULTS (all doses in mmole/kg unless otherwise stated)									
#	Report #	species/ strain	test substance	Mortality reached?	max non- lethal dose	min lethal dose	LD ₅₀	times of death	mult of clinical dose*
29	1101/05/91/027	mouse	MP-1177/10T	yes	10	20	25	5/14 5 min 9/14 2-8 days	8
30	1101/05/92/003	mouse	MP-1177/10	yes	0**	<24	28	12/15 <30 min 2/15 4-24 hr 1/15 11 days	-
31	1101/05/92/031A	mouse	MP-1177	yes	0**	<8	9.8	5 high dose ♂ s 1-24 hr 30/35 1-7 days	-
32	1101/05/94/008	mouse	MP-1177/10	yes	14	16	-	16/17 <30 min 1/17 4-24 hr	12
33	1101/05/93/021	mouse	Optimark, Magnevist	yes, yes	14, 4	16, 10	20.5, 4.7	20/23 <30 min 1/23 2-4 hr 2/24 2-7 days 17/17 <30 min	12, 3
34	1270/05/93/038	mouse	Optimark, Magnevist	yes, yes	0**, 2	<16, 4	24.0, 4.9	17/24 <30 min 1/17 4-24 hr 6/24 2-5 days 10/10 <30 min	-, 2
35	1101/05/90/024-E	mouse	MP-1177 plus MP-1196 and Ca	yes	0**-31	<16-36	<6-33	32/34 <30 min 1/34 4-24 hr 1/34 5 days	8-26
36	1101/05/92/046	mouse	MP-1196	yes	0**	<10	15.2	10/20 <30 min 3/20 2-24 hr 6/20 2-4 days	-
37	1101/05/92/031B	mouse	2-methoxyamine (an impurity)	yes	0**	<6	8	26/26 <30 min	-

(Table continued next page)

Summary of Single Dose Studies-Optimark-RESULTS (continued) (all doses in mmole/kg unless otherwise stated)										
#	Report #	species/ strain	test substance	Mortality reached?	max non- lethal dose	min lethal dose	LD ₅₀	times of death	mult of clinical dose*	
38	1101/05/93/018-E	mouse	MP-1177/10-4 lots (incl from 30. and 33.)	yes	0**	<20	♂ 22.7-26.0 ♀ 24.2,20.4	54/58 <30 min 2/58 2-24 hr 2/58 1-2 days	-	
39	1101/05/93/050	mouse	MP-1177/10-3 lots (test manufact change)	yes	0**	<16	20.9-23.6	54/58 <30 min 4/58 4-7 days	-	
40 ¹	1101/05/94/028	rat	MP-1177/10	no	>15***	>15	-	0	-	
41	1101/03/95/014-E	rat	MP-1177/10	no	>16***	>16	-	0	-	
42 ¹	1101/05/92/004	rat	MP-1177/10	yes	50 umole/kg	150 umole/kg	166 umole/kg	9/11 <1 hr 1/11 4-24 hr 1/11 9 days	see rev	
43	Bozo B-2481	rat	MP-1196	no	>4***	>4	-	0	-	
44 ¹	1101/05/91/032	beagle dog	MP-1177/10T	no	>12***	>12	-	0	-	
45	Bozo B-2483	beagle dog	MP-1196	no	>4***	>4	-	0	-	
46	1101/05/94/016-E	mouse	MP-1177/10	to be reviewed in NDA 20-976						

*multiple based on body surface area comparison of animal Maximum non-lethal dose and human dose of 0.1 mmole/kg
 **death at all doses
 ***death not observed at any dose
¹ reviewed in detail in text

Summary of Single Dose Toxicology

Single Dose and Multiple Dose studies will be summarized together at the end of the Multiple Dose Toxicology Section.

Multiple Dose Toxicology Studies

47. Title: MP-1177/10T: Toxicity Study by Intravenous Administration to CD Rats for 4 Weeks Followed by 4-Week and 8-Week Reversibility Periods

Study #: 1101/05/91/029

Species/Strain/Source: rat/CD strain

Sex/age/body weight: male and female/42-49 days/188-241 g

Dose information:

Formulation: MP-1177/10T

Concentration(s): all 0.5 M except 0.1 mmole/kg group which received 0.05 M

Dosages: 0,0,0.1, 0.6, 3.0 mmole/kg/day 7 days/week for 4 weeks

Route of administration: iv

Volume of administration: 0, 6.0, 2.0, 1.2, and 6.0 ml/kg/day respectively.

Rate of administration: not given

Study design and schedule:

Grp	Treatment	Dose		Number of animals per group at designated termination times (days after the 28 th dose)					
				0		28		56	
		mmole/kg	multiple*	♂	♀	♂	♀	♂	♀
1	untreated control	0	0	10	10	5	5	5	5
2	saline control	0	0	10	10	5	5	5	5
3	MP-1177/10T	0.1	0.2	10	10	5	5	5	5
4	MP-1177/10T	0.6	1.0	10	10	5	5	5	5
5	MP-1177/10T	3.0	5	10	10	5	5	5	5

*Dose multiple based on body surface area comparison (mmole/m²)

PROCEDURE	TIME OF PROCEDURE /DATA COLLECTION
Acclimation	for eight days prior to initial dose
Dose administration	daily by iv injection for 28 days
Mortality	during acclimation/recovery periods-daily during treatment-2 times daily
Toxic Signs	during acclimation/recovery periods-daily during treatment-2 times daily
More detailed examination incl palpitation	weekly
Body Weight	once during acclimation, the day of treatment, and twice weekly thereafter
Food Consumption	weekly (group values)
Water Consumption	3 days/week
Ophthalmoscopy	screened during acclimation, all animals on day 26, all recovery animals prior to 4- week termination, remaining recovery animals prior to 8-week termination
Hematology	prior to dosing -on day 25 of trtmnt for non-recov rats
Clinical Chemistry	-after 3 wks of recov for 4-wk recov rats -after 7 wks of recov for 8-wk recov rats
Urinalysis	for 2 days -during 3 rd wk of trtmnt for non-recov rats -during 3 rd wk of recov for 4-wk recov rats -during 7 th wk of recov for 8-wk recov rats
Termination	0,28 and 56 days following final dose by carbon dioxide asphyxiation
Gross pathology exam	on day of scheduled termination
Organ weights	on day of scheduled termination
Tissue preservation	on day of scheduled termination
histological work-up and microscopic exam	following tissue preservation

Results:

Note: Since slight differences were sometimes found between saline and untreated controls, treatment groups will be compared to the saline controls in the review of this study.

Mortality:

No deaths attributed to treatment.

Toxic signs and physical exams:

Erythema, thickening, ulceration, encrustations, and/or exfoliation at injection site beginning day 11 and persisting through treatment. NOAEL=0.1 mmole/kg
Generalized hair loss, scabbing, or encrustation of the skin observed in males only. ♂ NOAEL=0.1 mmole/kg, ♀ NOAEL=3.0 mmole/kg

Fast or irregular breathing before dosing perhaps a learned response in 4/20 high dose males. NOAEL=0.6 mmole/kg

Dry abrasive areas in sacral/lumbar region in 2/20 males in high dose group. NOAEL=0.6 mmole/kg

Piloerection and/or ungroomed appearance in males. NOAEL=0.1 mmole/kg

Body Weight, Food Consumption, and Water Consumption data are in the table on the page after next.

Body weight:

Decreases in body weight values were observed in saline controls compared to untreated controls (13% difference). Therefore, results of treatment will be compared to saline controls.

During the 4-week dose administration period; high dose males and females gained 33% and 19% less weight than saline control males and females respectively. NOAEL=0.6 mmole/kg.

During the two 4-week recovery periods combined (total of 8 weeks), high dose males and females gained 16% and 79% more body weight than saline control males and females respectively. High dose males still weighed about 10% less than controls; however, their weight gain is interpreted as recovery. High dose females gained so much weight they outgrew all other groups. At the end of the 8-week recovery period, they weighed >15% more than saline controls.

No effects on body weight were observed in 0.1 and 0.6 mmole/kg dose groups.

Food Consumption:

During the 4-week treatment period, food consumption in high dose animals was only slightly depressed while food conversion efficiency was more dramatically depressed, especially in males. NOAEL=0.6 mmole/kg

During the two 4-week recovery periods, food consumption was only slightly elevated in high dose animals; however, food conversion efficiency was more dramatically improved, especially in females.

No effects on food consumption or food conversion efficiency were observed in 0.1 and 0.6 mmole/kg dose groups.

Water Consumption:

No effects of treatment on water consumption; it is noted that saline controls consumed 33% more water than untreated controls during the 8-week recovery period.

Ophthalmoscopy:

Focal corneal opacities observed after 3 weeks of high dose treatment.
NOAEL=0.6 mmole/kg.

Thick dark brown mucous discharge observed after 3 weeks of high dose treatment. NOAEL=0.6 mmole/kg.

Three weeks after the final dose, animals recovered from these effects.

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Percent Positive (↓) or Negative (↑) Change in Mean Body Weight, Food Consumption and Water Consumption from Specified Control									
Time Period	Compared Controls	Body Weight Gain		Food Consumption		Food Conversion Efficiency		Water Consumption	
		♂	♀	♂	♀	♂	♀	♂	♀
(4 wks)	saline vs untreated	14% ↓ (.05)	13% ↓	10% ↓	2% ↓	6% ↓	11% ↓	3% ↑	12% ↓
	high dose vs saline	33% ↓ (.01)	19% ↓	8% ↓	4% ↓	29% ↓	15% ↓	4% ↓	3% ↓
(4 wks)	saline vs untreated	10% ↑	3% ↑	2% ↓	0%	12% ↑	6% ↑	-	-
	high dose vs saline	26% ↑	34% ↑	5% ↑	2% ↑	22% ↑	32% ↑	-	-
(8 wks)	saline vs untreated	1% ↑	15% ↑	2% ↓	3% ↑	7% ↑	19% ↑	33% ↑	8% ↑
	high dose vs saline	16% ↑	79% ↑ (.01)	4% ↑	8% ↑	9% ↑	24% ↑	10% ↓	7% ↓

(.05) (.01) significantly different from specified control by Bartlett's test followed by Behrens-Fisher or Dunnett's
 *Recoveries presented for the 1st 4 weeks and entire 8-week period

Hematology:

Reversible decreases in RBCs, hemoglobin, and packed cell volume and increases in leucocytes and platelets were observed at 3.0 mmole/kg in both sexes and 0.6mmole/kg in males.

Hematology (Study #: 1101/05/91/029)						
Effect	Frequency, Degree Duration or Time of Effect	Dose Response?	NOAEL			
			After 25 days of treatment		After 3 wks recovery	After 7 wks recovery
			(mmole/kg)	as multiple of clinical dose ([dose]/m ² basis)		
↓ packed cell volume		yes	♂ 0.1 ♀ 0.6	0.2 1	0.6 3.0	3.0 3.0
↓ hemoglobin		yes	♂ 0.1 ♀ 0.6	0.2 1	0.6 0.6	3.0 3.0
↓ RBCs		yes	♂ 0.1 ♀ 0.6	0.2 1	0.6 3.0	3.0 3.0
↑ Total leucocytes		high dose only	♂ 0.6 ♀ 0.6	1 1	0.6 3.0	3.0 3.0
↑ platelets		high dose only	♂ 0.6 ♀ 0.6	1 1	0.6 0.6	3.0 3.0
↓ activated partial thromboplastin time	slight	high dose only	♂ 0.6 ♀ 3.0	1 5	0.6 3.0	3.0 3.0
poikilocytes present		yes	♂ 0.6 ♀ 3.0	1 5	3.0 3.0	3.0 3.0
spherocytes present		yes	♂ 0.6 ♀ 0.6	1 1	3.0 3.0	3.0 3.0

Clinical Chemistry:

Reversible changes in AST, LDH, triglycerides, and cholesterol were observed primarily at 3.0 mmole/kg in males. Reversible changes in phosphorus were observed in males and females at all doses (except 0.1 in females).

Clinical Chemistry (Study #: 1101/05/91/029)						
Effect	Frequency, Degree Duration or Time of Effect	Dose Response?	NOAEL			
			After 25 days of treatment		After 3 wks recovery	After 7 wks recovery
			(mmole/kg)	as multiple of clinical dose ((dose)/m ² basis)		
↓ AST	50% ↓ at 3.0	Yes	♂ 0.6 ♀ 0.0	1 0	0.1 3.0	3.0 3.0
↓ LDH	15% ↓ at 3.0	high dose only	♂ 0.6 ♀ 3.0	1 5	3.0 3.0	3.0 3.0
Δ triglycerides	♂ 40% ↓ at 3.0, 25 d trtmnt ♀ 100% ↓ at 3.0, 3 wk recov	no	♂ 0.6 ♀ 3.0	1 5	0.1 0.6	3.0 3.0
↓ cholesterol	30% ↓ at 3.0	high dose only	♂ 0.6 ♀ 3.0	1 5	3.0 3.0	3.0 3.0
↓ phosphorus	23% ↓ at 3.0	yes	♂ 0.0 ♀ 0.1	0 0.2	3.0 3.0	3.0 3.0

Urinalysis:

Positive and negative alterations in urine electrolytes (Na, K, and Cl) were found during treatment and at the first recovery period but appeared to normalize by the second recovery period. (Note that serum electrolyte values were normal at all time points.)

Slight decreases in urine pH (0.5 pH units) were found at all time points (except in 8-week recovery females) in the high dose group. Urine crystal count was decreased in males through the second recovery period; urine crystals were not measured in females. Spermatozoa count was decreased in the 3.0 mmole/kg males at all time points.

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Organ Weights:

Testis, epididymis, and prostate weights (absolute and bodyweight-relative) were irreversibly reduced in the 3.0 mmole/kg dose group compared to controls. Kidney weights (absolute and bodyweight-relative) were irreversibly increased. However, examination of mean data by the reviewer revealed that fluctuations in kidney weights in both sexes during the recovery periods suggested recovery may have been starting to take place.

Organ Weights (Study #: 1101/05/91/029)						
Effect	Frequency, Degree Duration or Time of Effect	Dose Response?	NOAEL			
			After 4 weeks treatment		After 4 wks recovery	After 8 wks recovery
			(mmole/kg)	as multiple of clinical dose ([dose]/m ² -basis)		
↓ testis weights	45-55% abs and rel (all time points)	high dose only	♂ 0.6	1	0.6	0.6
↓ epididymis weights	25-40% abs and rel (all time points)	high dose only	♂ 0.6	1	0.6	0.6
↓ prostate weights	10-25% abs and rel (all time points)	high dose only	♂ 0.6	1	0.6	0.1
↑ kidney weights	10-30% abs and rel (all time points)	high dose only	♂ + ♀ 0.6	1	0.6	0.6

Gross Pathology:

MP-1177/10T produced small/flaccid testes and epididymides in the 3.0 mmole/kg group which was found to be irreversible after 8 weeks. Reversible thickening of the stomach glandular mucosa and dark areas within the mucosa were found in males receiving 3.0 mmole/kg/day; this effect was not observed in females.

Macroscopic findings (study #: 1101/05/91/029)					
Effect	Dose Response?	NOAEL			
		After 4 weeks treatment		After 4	After 8
		(mmole/kg)	As multiple of clinical dose ([dose]/m ² basis)	Wks recovery	Wks recovery
Small/flaccid testes	High dose only	♂ 0.6	1	0.6	0.6
Small epididymides	High dose only	♂ 0.6	1	0.6	0.6
Thickening of the stomach glandular mucosa	High dose only	♂ 0.6	1	3.0	3.0
		♀ 3.0	5	3.0	3.0
Punctate dark areas within mucus of glandular mucosa	High dose only	♂ 0.6	1	3.0	3.0
		♀ 3.0	5	3.0	3.0

Microscopic Pathology:

Reversible vacuolization of the kidney proximal tubules observed at 0.6 and 3.0 mmole/kg. Irreversible histopathology of male reproductive organs observed at 3.0 mmole/kg. Punctate dark areas of stomach mucosa found to be composed of RBCs.

Histopathology (Study #: 1101/05/91/029)						
Organ	Effect	Dose Response?	NOAEL			
			After 4 weeks treatment		After 4 wks recovery	After 8 wks recovery
			(mmole/kg)	as multiple of clinical dose ([dose]/m ² basis)		
kidney	macro- and micro- vacuolization of the proximal tubules	yes	♂ 0.1	0.2	3.0	3.0
			♀ 0.1	0.2	3.0	3.0
testes	degeneration of germinal epithelium	high dose only	♂ 0.6	1	0.6	0.6
testes	spermatid giant cells	high dose only	♂ 0.6	1	0.6	0.6
epididymides	markedly reduced sperm count, germ cells present	high dose only	♂ 0.6	1	0.6	0.6
stomach	punctate dark areas of the mucosa composed of RBCs	high dose only	♂ 0.6	1	3.0	3.0
			♀ 3.0	5	3.0	3.0

Reviewer comments:

Major effects found primarily in the high dose group (3.0 mmole/kg) in this 4-week multiple dose toxicity study with 4 and 8 week recovery periods were:

1) reversible decreases in body weight gain in males and females and excessive body weight gain in high dose females during recovery. Body weight changes appeared to be due to changes in food conversion efficiency. NOAEL=0.6 mmole/kg

2) irreversible toxicity to the male reproductive system as indicated by: decreased size and weight of testes, epididymides, and prostate, degeneration of the germinal epithelium of the testes, reduced sperm count in the epididymides, and decreased urinary spermatozoa count. NOAEL=0.6 mmole/kg

3) suggestions of kidney toxicity including

- a. irreversible elevation of kidney weight, NOAEL=0.6 mmole/kg
- b. decreased urinary pH NOAEL 0.6 mmole/kg
- c. reversible vacuolization of the proximal convoluted tubules of the kidney, NOAEL= 0.1 mmole/kg

Reversible thickening of the stomach mucosa with bleeding in high dose males is noted.

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48. Title: A Subchronic (4-Week) Toxicity Study of MP-1177/10T in the Dog via Intravenous Injection with an 8-Week Recovery Period

Study #: 1101/05/91/033

Species/Strain/Source: dog/beagle

Sex/age/body weight: ♂ /6.2-8.2 kg and ♀ /5.5-6.9 kg

Dose information:

Formulation: MP-1177/10T

Concentration(s): 0.5 M

Dosages: 0, 0.1, 0.6 and 3.0 mmole/kg (0, 0.5, 3, and 15 times the proposed human dose of 0.1 mmole/kg based on a body surface area comparison)

Route of administration: iv

Volume of administration: 6.0, 0.2, 1.2, and 6.0 ml/kg respectively

Rate of administration: 500 ml/hr or entire dose in 5 min, 10 sec, 1 min, and 5 min respectively

Study design and schedule:

Experimental Outline:

Group	Test Substance	Dose Level mmol/kg/day	Dose Volume ml/kg/day	Number of Animals											
				Total		Clinical Laboratory Evaluations ^a						Terminal Sacrifice		Recovery Sacrifice	
						Pretest		Termination		Recovery					
				M	F	M	F	M	F	M	F	M	F	M	F
I	Control	0	6.0	5	5	5	5	5	5	2	2	3	3	2	2
II	MP-1177/10T	0.1	0.2	5	5	5	5	5	5	2	2	3	3	2	2
III	MP-1177/10T	0.6	1.2	5	5	5	5	5	5	2	2	3	3	2	2
IV	MP-1177/10T	3.0	6.0	5	5	5	5	4	4	2	2	2	2	2	2

^aClinical laboratory studies include hematology, clinical chemistry and urinalysis.

M - Male, F - Female.

PROCEDURE	TIME OF PROCEDURE /DATA COLLECTION
Acclimation	4 weeks prior to initial dose
Dose administration by infusion pump (control, high dose) or bolus (low and mid doses)	daily for 28-30 days
Observe for mortality	twice daily
Observe for clinical signs	twice daily
Conduct detailed physical exam	pretest and weekly thereafter
Ophthalmoscopic exam	pretest, all animals a few days before initial termination, recovery animals a few days before final termination
Body weight	pretest, weekly thereafter, and at termination (fasted)
Food Consumption	estimated 4 times a week beginning 1 week before initial dose and continuing until termination
Hematology	pretest and 1 to 5 days prior to initial and recovery terminations
Clinical chemistry	
Urinalysis	
Exsanguination under sodium pentobarbital anesthesia	0 and 8 weeks following the final dose
Organ weights	on day of scheduled termination
Gross pathological exam	
Tissue preservation	
Histopathological work-up and microscopic exam	following tissue preservation

Results:Mortality:

Two deaths occurred during the study:

1 high dose male died on day 13 of the treatment period

1 high dose female was terminated in moribund condition on day 15 of treatment

Both deaths were attributed to treatment.

Clinical Signs and Physical Exams:

Treatment-related effects were only observed in 3 animals:

Lethargy, uncoordinated movements, tremors, irregular gait, reduced food

consumption, and watery, discolored stools were observed in the 2 high dose animals that died.

A high dose surviving male appeared thin and dehydrated during the 3rd and 4th weeks of the study.

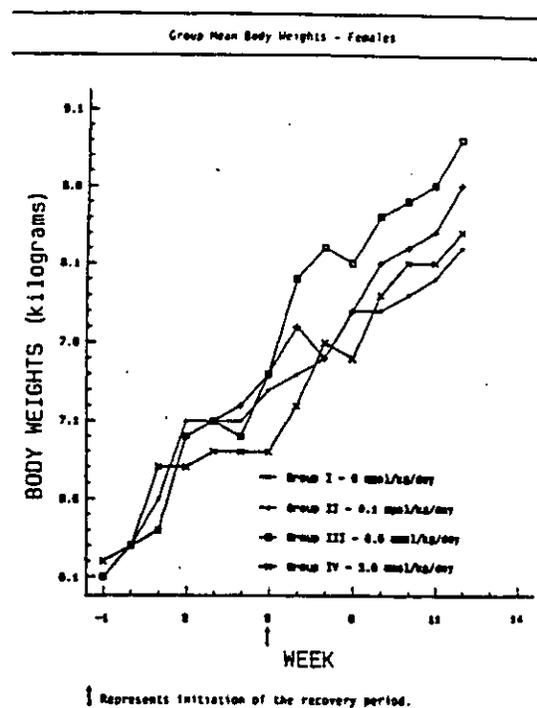
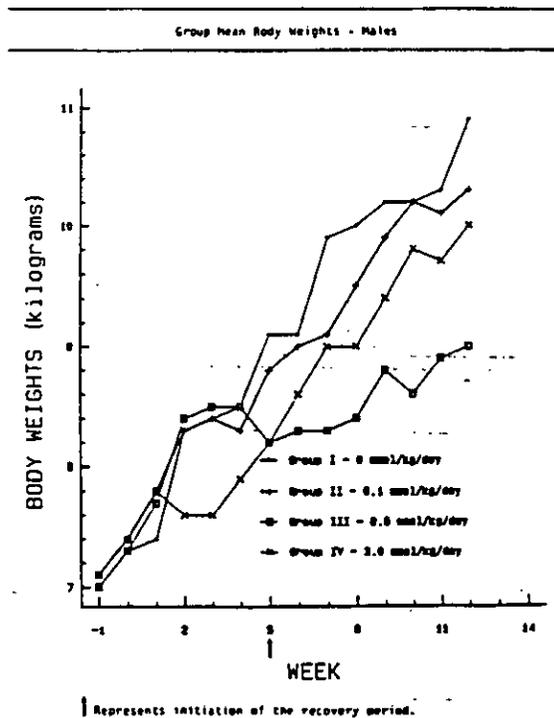
Ophthalmoscopy:

No effect.

Body weight:

It appears from the Sponsor's graphs that 3.0 mmole/kg dose males lost weight during part of the treatment period while males in other dose groups gained weight. 0.6 mmole/kg males lost weight at the end of the treatment period and appeared to gain weight at a reduced rate during recovery.

An effect on body weight was not found in females.



Food Consumption:

Food consumption was not measured and normalized to body weight. Food consumption was recorded as visual estimates of the amount of food consumed using the following key:

KEY TO FOOD CONSUMPTION REPORTING CODES

- 0 = No observable food consumed.
- 1 = Up to and including 1/4 consumed.
- 2 = Up to and including 1/2 consumed.
- 3 = Up to and including 3/4 consumed.
- 4 = Up to but not including the entire amount consumed.
- 5 = Total amount consumed.

Using this method, the Sponsor claimed that the high dose males consumed less food during treatment than controls. No differences in food consumption were apparent during the recovery period.

Weighing the food would have provided better data.

Hematology:

No effects.

Clinical chemistry:

The Sponsor reported that alkaline phosphatase levels were elevated in females receiving 28 daily doses of 3.0 mmole/kg. The Sponsor noted that the elevated mean was the result of 1 animal with a value 3 times the mean of the rest in the group which was considered coincidental. The reviewer feels that although most changes were still within historical control values, a slight trend upward seemed to be apparent for both sexes at all doses after 28 days of dosing. Recovery of the elevations were observed 8 weeks following the last dose.

The Sponsor concluded that there were slight elevations in chloride levels in the 0.6 and 3.0 mmole/kg groups at the end of the treatment period. The reviewer checked the chloride values and concluded that the test article had no effect on chloride values.

Dose-related decreases in serum phosphorus concentration were found in males and females after 28 days of dosing. These decreases were still within the normal ranges for phosphorus and showed recovery at 8 weeks after the last dose. The Sponsor did not consider this effect to be biologically significant.

Test	Dose mmol/kg	Males			Females		
		pretest	1 st Term	Recov Term	Pretest	1 st Term	Recov term
ALK PHOS (IU/L)	0	140	133	81	102	84	67
	0.1	116	131	84	113	147	121
	0.6	142	176	126	113	143	109
	3.0	129	158	157	156	202	165
Cl ⁻ (mEq/L)	0	111	113	110	112	113	111
	0.1	112	114	112	113	115	114
	0.6	111	115**	113	113	116	114
	3.0	113	116**	113	113	116	114
PHOS (mg/dl)	0	6.7	6.5	5.9	6.5	6.1	5.5
	0.1	6.6	5.5	5.7	6.3	5.1*	5.2
	0.6	6.7	4.5*	5.8	6.3	4.2**	5.4
	3.0	6.9	4.1**	4.9	6.7	3.7**	4.6

Historical control values-Beagle dog				
	Male		Female	
	mean	range	mean	range
ALK PHOS (IU/L)	114	39-191	119	47-186
chloride (mEq/L)	110	102-119	111	101-121
PHOS (mg/dL)	5.89	4.08-7.77	5.76	3.81-7.33

The female dog sacrificed in moribund condition on the 15th day of treatment may have died of renal failure as shown by the following laboratory values. It appears that cardiac or liver damage may have contributed to toxicity. It would have been useful to know the water consumption and urine volume of this animal to determine if dehydration could have been a contributing cause.

Abnormal Parameter	♀ Normal value (range)	Found in ♀ Dog #4780
AST (SGOT)	31 (20-47)	126
ALT (SGPT)	29 (17-45)	72
ALK PHOS	119 (47-186)	30
BUN	15 (8.5-21.7)	OFF SCALE
S CREAT	0.8 (0.7-1.0)	3.2
Ca ⁺⁺	10.5 (9.7-11.4)	3.8
PHOS	5.7 (3.8-7.3)	13.3

Other clinical chemistry values for this animal were normal.

Urinalysis:

Urinary calcium values dropped to 0 in almost all males and females in the 0.6 and 3.0 mmole/kg dose groups (NOAEL=0.1 mmole/kg) after 4 weeks of treatment. This effect was reversed 8 weeks following the final injection.

Organ weights:

Optimark produced a dose related increase (60 to 80% in high dose males and females) in kidney weights after 4 weeks of treatment (NOAEL=0.1 mmole/kg). Partial recovery was observed 8 weeks following the final dose (25% increase in high dose males and females, NOAEL=0.1 mmole/kg).

Gross pathology:

Discoloration (tan, brown) of the kidneys was observed in high dose animals (NOAEL=0.6 mmole/kg) after 4 weeks of treatment. This effect was reversed 8 weeks following the final injection.

Histopathology:

Cytoplasmic vacuolation of the epithelium of the convoluted tubules of the epithelium was observed in mid and high dose animals (NOAEL=0.1 mmole/kg) after 4 weeks of treatment. The severity was dose related. This effect was reversed 8 weeks after the final dose.

Vacuolation of the convoluted tubules was also observed in kidneys of the animal that died on test and the animal that was terminated in moribund condition.

Reviewer comments:

Major effects found in this 4-week multiple dose toxicity study in dogs with an 8-week observation period were:

- 1) mortality in the 3.0 mmole/kg group on days 13 and 15 of treatment (Max nonlethal dose=0.6 mmole/kg). A possible cause of death was kidney failure but clinical chemistry values suggested that heart parameters or liver parameter changes may have contributed.
- 2) reduced body weight gain in 0.6 and 3.0 mmole/kg males but not females with at least partial recovery at 8 weeks (NOAEL=0.1 mmole/kg).
- 3) suggestions of evidence of kidney toxicity including
 - a. elevated BUN and creatinine in a moribund high dose female.
 - b. other clinical chemistry and urinalysis changes that may or may not be indicative of kidney toxicity including irreversible elevation in alkaline phosphatase (NOAEL=0), reversible elevation in phosphorus (NOAEL=0), reversible cessation of urinary calcium elimination (NOAEL=0.1 mmole/kg).
 - c. 60 to 80% increase in kidney weights (NOAEL=0.1 mmole/kg) partially recoverable 8 weeks after the treatment period.
 - d. reversible discoloration of the kidneys (NOAEL=0.6 mmole/kg)
 - e. reversible vacuolization of the convoluted tubules of the kidney (NOAEL=0.1).

Summary of Single and Multiple Dose Toxicology

Two of the multiple dose studies and 4 of the single dose toxicology studies submitted were considered pivotal and were therefore reviewed in detail. Note that pertinent data from all single dose toxicity studies are summarized in tables at the end of the single dose toxicology section of this review.

The design of the multiple dose toxicity study in rats included 4 weeks of daily iv doses and terminations of animals at 0, 4 and 8 weeks following the final dose. The design of the multiple dose toxicity study in dogs included 4 weeks of daily iv doses and terminations of groups of animals at 0 and 8 weeks following the final dose. The times of terminations were set to test for early and late effects and reversibility of effects.

Two of the 4 single dose studies were selected for review because they were conducted in the same species (rats and dogs) as the multiple dose studies. The other 2 single dose studies were selected for review because they addressed specific issues of importance:

-the effects of varying levels of MP-1196 (versetamide) and calcium on the toxicity of MP-1177 (gadoversetamide) in mice. These ingredients are present in the final formulation at 10 molar percent.

-the effects of intracisternal administration of Optimark on toxicity to the brain of rats. Optimark is indicated for use in imaging brain lesions with disrupted blood brain barrier which means normal brain tissue in close proximity to the lesion may be exposed to Optimark.

The major findings after 4 weeks of daily dosings (up to 3.0 mmole/kg/day) of MP-1177/10T to rats were reversible body weight loss (NOAEL=0.6 mmole/kg); irreversible toxicity to the male reproductive system as evidenced by reduced weight of reproductive organs and histopathological changes (degeneration of the germinal epithelium of the testes, reduced sperm count and germ cells present in epididymides) (NOAEL=0.6 mmole/kg); signs of kidney toxicity such as irreversible increase in kidney weight, urinary pH changes, and reversible dose-related vacuolization of the proximal convoluted tubules (NOAEL=0.6, 0.6, and 0.1 mmole/kg respectively). The data from this multiple dose study suggested that a margin of safety does not exist; therefore, the Sponsor conducted an acute toxicity study with special emphasis on findings from this study.

In the single dose rat toxicity study, only males were tested; the reviewer considers this adequate since: effects on the male but not the female reproductive system were found in the 4 week multiple dose study, and males were more sensitive than females to effects other than reproductive effects. Following single doses of MP-1177/10 up to 15 mmole/kg, effects were not observed on body weight and the male reproductive system 24 hours and 14 days after dosing. Effects on the kidneys were found: grossly pigmented kidneys and moderate vacuolization of the proximal tubules 24 hours after dosing (NOAEL=0.5 mmole/kg); however, signs of recovery were observed 14 days after dosing although recovery was not complete (NOAEL=5.0 mmol/kg or 8 times the clinical dose). This study would have been improved if the interim sacrifice had been conducted 3 days after dosing and clinical chemistries had been measured.

The major findings after 4 weeks of daily dosings (up to 3.0 mmole/kg/day) of MP-1177/10T to dogs were lethality (max non-lethal dose 0.6 mmole/kg or 3 times the human dose); body weight loss in males (NOAEL=0.1 mmole/kg or no margin of safety); increased alkaline phosphatase (irreversible 8 weeks following the final dose) and decreased phosphorus (reversible) (NOAEL=0); and toxicity to the kidneys as evidenced by reversible cessation of calcium elimination as measured by urinary calcium (NOAEL=0.1 mmole/kg), dose related increases in kidney weights showing

partial recovery 8 weeks after the final dose (NOAEL=0.1 mmole/kg), discoloration of kidneys, and reversible vacuolization of the proximal tubular epithelium (NOAEL of kidney effect=0.1 mmole/kg or no margin of safety). Blood chemistry values for the dog sacrificed in moribund condition on the 15th day of daily dosing at 3.0 mmole/kg suggested that renal failure combined with cardiac or liver damage may have been the cause of morbidity.

Note that effects on the male reproductive system found in rats was not observed in dogs.

Single dose toxicity testing in dogs showed that doses up to 12 mmole/kg had no effect on dogs except a slight elevation alkaline phosphatase and a slight decrease in phosphorus levels. Even though these effects were observed at most dose levels, they were still within historical control values. Note that effects on kidney were not observed in this study.

Regarding the study of the effects of MP-1196 (versetamide) and calcium on the toxicity of MP-1177 (gadoversetamide) in mice, it seems that the objective of such a study should have been to find out the optimal doses of each of these in the final formulation. However, this study did not justify the levels (10%) of MP-1196 and Ca currently added to the MP-1177/10 final formulation. It appeared from the data, that safety was maximized when the ratio of calcium to MP-1196 was 1. However, it also appeared that the optimal ratio of MP-1196 and calcium to MP-1177 may be <1% , not 10%.

Regarding the study of the effects of intracisternal administration of Optimark on toxicity to the rat brain, the reviewer found that it was difficult to compare rat brain exposure following an intracisternal dose to possible brain exposure in humans following an iv dose without making a lot of assumptions. However, it was found that the margin of safety for clinical signs of neurotoxicity (such as dyspnea, hypoactivity, convulsions, tremors, rearing pawing) is at least 21. This safety factor was estimated using conservative assumptions in terms of safety about the brain kinetics of MP-1177 in rats and humans.

The Sponsor conducted many single dose studies in mice which are summarized in tables at the end of the single dose section. Several observations of data in the tables are noted: most deaths in mice occurred rapidly (<30 min) although a few deaths in each study appeared to be delayed (for 2 to 24 hrs or for days); the incidence of death was dose-related; the time of death did not appear to be dose-related. Most animals surviving the first 30 min returned to normal within a few hours as measured by clinical signs; however, hypoactivity and unkempt appearance persisted in animals exhibiting delayed death. Males seemed to be more sensitive to MP-1177/10 than females; however, since differences were not extreme, the reviewer decided to report results of males and females combined.

It would be interesting to know if delayed deaths in mice could be prevented with clinical intervention. Knowing the answer to this might be relevant for humans (example: accidental overdosing in clinically compromised patients).

The following table compares maximum non-lethal doses of the 3 currently approved gadolinium agents and Optimark:

Maximum Non-lethal Doses after Single Administration of Gadolinium Agents				
Species	Estimated Multiple of Human Dose of 0.1 mmole/kg*			
	Magnevist	Omniscan	ProHance	Optimark
mouse	7-11	15-17	2.5	8-12
rat	13-42	>8, >33	>16**	>27**
rabbit	53		>20 *******	
dog	>100**		>15**	>60**

*body surface area comparison
 **the minimum lethal dose was not reached in the study. The listed value is the highest dose in a study in which deaths were not observed at any dose level.
 ***lethality to pregnant animals within 24 hrs of the 1st dose in a reproduction study

Carcinogenicity Statement

The Sponsor states, "Carcinogenicity studies for this product are not necessary since Optimark will not be administered regularly over a substantial portion of a patient's lifetime but used only a few times during the life of the patient."

Reproductive Toxicology-individual studies reviewed by David E. Bailey, Ph.D.

54. A Study Of The Effect of MP-1177/10 On Fertility And General Reproductive Performance On The Rat. Study No. MLL 12/931339. Laboratory [

In-Life Study Dates, June 17-December 2, 1992.

Report Dated March 20, 1995. Lot Number B1658p57. In Compliance With GLP. Report In Volume 1.22, pp 7-271.

Sponsors Study 1101/05/92/017

Design: This study was designed to assess the effects of MP-1177/10 (Optimark) on fertility and general reproductive performance on male and female rats when administered daily to F₀ generation rats. Males were treated from 9 weeks prior to mating and during the 20 day cohabitation period. Females were treated from 2 weeks prior to mating through to either caesarean sectioning at day 20 of presumed gestation or to weaning of their respective F₁ litters. One-hundred forty [CD(SD) BR VAF/Plus] male rats weighing 188-221 g and 7-8 weeks of age and the same number and strain of female rats weighing 205-245 g and 8-10 weeks of age, were equally distributed among 4 treatment groups, and administered daily

intravenous doses in the tail vein, of either saline or Optimark. A saline dosage of 4.0 mL/kg/day and Optimark dosages of 0.1, 0.5 and 2.0 mmol/kg/day were used. Dosages were equivalent to volumes of 4.0, 0.2, 1.0 and 4.0 mL/kg/day for Control through high dose, respectively, and were injected at a rate of 1 mL/minute. Dosages were selected based on results from an earlier 4 week study with doses ranging from 1.0-4.9 mmol/kg/day, where minimal growth and weight depression were observed at 2.0 mmol/kg/day. Due to severe toxicity seen in males in the 2.0 mmol/kg/day group during the premating treatment period, the study was modified. In Part I of the revised study, the control group, the 0.1 and 0.5 mmol/kg/day groups continued to follow the protocol as indicated. In Part II of the revised study, treatment of the F₀ males of the 2.0 mmol/kg/day group was discontinued after 7 weeks, and the F₀ females were never treated at 2.0 mmol/kg/day.

Part I: Control, 0.1 and 0.5 mmol/kg/day groups.

On day 20 of presumed gestation, 20 F₀ females per group were sacrificed by CO₂ asphyxiation and uterine contents examined. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. The number and distribution of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded. All fetuses were weighed, sex recorded and pups preserved for possible future evaluation of visceral and skeletal alterations. Indices for pre-implantation loss and post-implantation loss were calculated. The other 15 F₀ dams per group were allowed to give live birth and nurse the F₁ offspring to weaning at 21 days post partum. All dams were observed daily for survival and clinical signs. Body weights were recorded on Days 0, 2, 4, 7, 8, 10, 12, 14, 18 and 20 of presumed gestation and again on days 0, 7, 14 and 21 post partum. Feed consumption values were recorded for the same intervals. The following indices were calculated for the F₀ dams: Gestation, Birth, Day 4 Viability and Weaning Viability.

F₁ and F₂ Generations:

Randomly selected F₁ offspring of 12/sex/ group were retained to maturity, paired (not with litter mates) and allowed to litter and rear the F₂ offspring to weaning. Dams were weighed on presumed gestation days 0, 3, 7, 10, 14, 17 and 20 then on days 0, 4, 7, 14 and 21 post partum. After birth, pups were weighed, individually identified by toe clip, sexed and weighed again on days 4, 8, 12, 16 and 21 post partum. Dams of the F₀ and F₁ generations were sacrificed by CO₂ asphyxiation at weaning of their pups on lactation day 21. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. All F₂ and the F₁ offspring not selected for the growth phase of the study were also sacrificed at weaning, weighed and evaluated at gross necropsy. Parameters observed in the F₁ generation during the pre-weaning period included: Surface Righting Reflex, Startle Reflex, Air Righting Reflex and Pupil Reflex. Parameters observed in the F₁ generation during maturation included date of vaginal opening in females and cleavage of the balanopreputial skinfold in males. Developmental and behavioral examinations included: Accelerating Rotarod Test, 'Actimat' Test, and Passive Avoidance Test. The following indices were calculated for the F₁ dams: Copulation, Fertility, Gestation, Birth, Day 4 Viability and Weaning Viability.

Part II: 2.0 mmol/kg/day.

After 7 weeks of treatment, the F₀ males were exhibiting signs of toxicity including mortality, and reduced body weight gain and food consumption. Treatment of these males was discontinued and 10 were immediately sacrificed. Gross necropsy and histopathologic evaluations were conducted. Following a 4 week recovery period, 12 males were cohabited with 12 untreated females for a 20 day period, after which the males and females were sacrificed and subjected to gross necropsy. This was equivalent to a total 7-week recovery period from cessation of treatment of these F₀ males to sacrifice. For the males, histological examination was limited to testes and epididymides, with the uterine contents of the females examined. Following an 8 week recovery period, another subgroup of 12 males were cohabited with 12 untreated females for a 20 day period, after which the males and females were sacrificed and subjected to gross necropsy. The total recovery period for the males of this subgroup was 11 weeks from cessation of treatment until sacrifice. For the males, histological examination was limited to testes and epididymides, with the uterine contents of the females examined.

Results:**Part I: Control, 0.1 and 0.5 mmol/kg/day groups.****F₀ Generation:**

No treatment-related deaths or clinical signs were observed in any group. There were no effects on body weight and food consumption of males and females seen in any of the groups. There were no effects related to treatment in the 0.1 and 0.5 mmol/kg/day on the F₀ dams or their untreated F₁ offspring.

F₁ and F₂ Generations:

Litter and fetal parameters were unaffected by treatment in the 0.1 and 0.5 mmol/kg/day groups. At 0.5 mmol/kg/day, F₁ litter weight at birth and through to weaning was reduced, compared to controls, with statistical significance ($p \leq 0.05$) continuing from lactation day 4 through to weaning. There were no treatment related effects on pre-weaning developmental reflexes nor post weaning behavioral tests of the F₁ and F₂ offspring as a result of treatment of the males and females of the F₀ generation. There were likewise no other behavioral, external, visceral or skeletal effects observed.

Part II: 2.0 mmol/kg/day

During the 7 week pre-mating treatment period, one F₀ male was sacrificed exhibiting treatment related effects of weight depression, decreased food consumption, periorbital discharge, hair loss, scabbing, poorly groomed coat and pale extremities. At necropsy small testes and enlarged cervical lymph nodes were observed. Body weight and food consumption were significantly decreased for the males of this group from week 3-7. With the toxicity that was observed, the

decision was made to discontinue treatment of the males of this group and 10 of them were sacrificed. Microscopic evaluation revealed dermal inflammation, hyperkeratosis, acanthosis and reduction of hair follicles of skin; vacuolation of proximal tubular epithelium of kidneys; reduction and degeneration of spermatocytes in the testes; and absence of spermatozoa in the caput, and reduction of spermatozoa in the cauda of the epididymides. The additional groups of 12 males that underwent recovery periods prior to mating also exhibited testicular and epididymal histologic effects. Fertility of the males was greatly decreased in both of the recovery groups. There was only 1/12 pregnancies in the 4 week recovery group resulting from copulation of 11/12 pairs of animals. In the 8 week recovery group, evidence of copulation was present only in 2/12 pairings, and with only 1/12 pregnancies.

SPONSOR'S CONCLUSION

The NOEL for fertility and reproductive performance was 0.5 mmol/kg/day. The NOEL for fetal growth and development was 0.1 mmol/kg/day.

REVIEWER'S COMMENT

Agree. The NOEL for fertility and reproductive performance in this study is 0.5 mmol/kg/day. The NOEL for growth and other fetal effects is 0.1 mmol/kg/day. Maternal toxicity was not observed at either dose.

APPEARS THIS WAY
ON ORIGINAL

55. A Study Of The Effect of MP-1177/10 On Pregnancy Of The Rat. Study No. MLL 17/930511. In-Life Study Dates, June 16-November 23, 1992. Report Dated March 20, 1995. Lot Number S92120-C. In Compliance With GLP. Report In Volume 1.23, pp 1-215.

Sponsor's Study 1101/05/92/022

Design: This study was designed to assess the maternal and fetal effects of MP-1177/10 (Optimark) when administered to pregnant rats on day 7-17 of presumed pregnancy. One-hundred-sixty timed-pregnant CD®(SD) BR VAF/Plus] female rats weighing 176-259 g and 8-10 weeks of age, were equally distributed among 4 treatment groups, and administered daily intravenous doses in the tail vein of either saline or Optimark on days 7 through 17 of presumed gestation. A saline dosage of 9.8 mL/kg/day and Optimark dosages of 0.1, 0.7 and 4.9 mmol/kg/day were used. Dosages were equivalent to volumes of 9.8, 0.2, 1.4, and 9.8 mL/kg/day for Control through high dose, respectively, and were injected at a rate of 1 mL/minute. Dosages were selected based on a preliminary dose range finding study with doses ranging from 1.0-4.9 mmol/kg/day, where retarded pup growth and minimal maternal weight depression were observed. All dams were treated days 7-17 of presumed gestation, with 20 pregnant dams sacrificed on day 20 of presumed gestation, and the remainder allowed to give live birth and nurse the offspring to weaning at 21 days post partum.

Part I: Caesarean Sectioning.

Design: All rats were observed daily for survival and clinical signs. Body weights were recorded on Days 0, 2, 4, 7, 8, 10, 12, 14, 18 and 20 of presumed gestation. Feed consumption values were recorded for the same intervals. Dams were sacrificed by CO₂ asphyxiation on day 20 of presumed gestation and uterine contents examined. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. The number and distribution of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded. All fetuses were weighed and evaluated for visceral and skeletal alterations and sex recorded. Indices for pre-implantation loss and post-implantation loss were calculated.

Results: No maternal deaths were observed. No treatment-related clinical signs were observed in any group. In dams of the 4.9 mmol/kg/day group, the only effects related to Optimark treatment included depression of maternal body weight gains and minimal decreases in food consumption during the treatment period only. Maternal effects on body weight and food consumption were not seen in the 0.1 and 0.7 mmol/kg/day groups. Litter and fetal caesarean sectioning parameters of number and distribution of corpora lutea, implantations, early and late resorptions, live and dead fetuses, fetal weight, visceral and skeletal alterations, sex ratio, and indices for pre-implantation loss and post-implantation loss were unaffected by treatment. The only fetal effects observed which are considered effects of Optimark treatment are retarded growth, abnormal liver lobation and delayed ossification or unossification of sternbrae in the 4.9 mmol/kg/day group. There were no other external, visceral or skeletal effects observed in any of the groups. No fetal effects were observed in the 0.1 and 0.7 mmol/kg/day groups.

PART I STUDY SUMMARY TABLE

PARAMETER	TREATMENT GROUP (Treated on days 7-17 of presumed gestation)			
	Control (9.8 mL/kg/day)	0.1 mmol/kg/day (0.2 mL/kg/day)	0.7 mmol/kg/day (0.4 mL/kg/day)	4.9 mmol/kg/day (9.8 mL/kg/day)
Dams Assigned	24	24	23	23
Not Pregnant	4	4	3	3
Live Litters	20	20	20	20
Mean Litter Corpora Lutea	14.6	16.0	14.6	15.1
Mean Litter Implants	13.1	14.9	13.0	14.1
Pre-Implant Loss (%)	12.2	6.4	11.4	6.2
Post-Implant Loss (%)	3.1	5.5	7.3	7.7
Total Group Live Pups	254	282	240	260
Mean Litter Live Pups	12.7	14.1	12.0	13.0
Mean Fetal Wt. (g)	3.75	3.71	3.77	3.59
% Males	45.8	45.9	48.7	45.9
Malformed (fetuses/litters)	2/2	2/2	0/0	1/1
Abnormal Liver Lobation (fetuses/litters)	3/3	3/3	1/1	10/9
Sternebrae Ossification Deficit (%)	31.5	32.9	33.7	55.5*

Key: * $p \leq 0.05$

Part II: Live Birth to Weaning.**F₀ Generation:**

Design: F₀ dams were treated only on days 7-17 of presumed gestation. All dams were observed daily for survival and clinical signs. Body weights were recorded on Days 0, 2, 4, 7, 8, 10, 12, 14, 18 and 20 of presumed gestation. Feed consumption values were recorded for the same intervals. Dams were allowed to deliver live litters and nurse the offspring to weaning. The following indices were calculated for the F₀ dams: Gestation, Birth, Day 4 Viability and Weaning Viability.

F₁ and F₂ Generations:

Design: Randomly selected F₁ offspring of 10/sex/ group were retained to maturity, paired (not with litter mates) and allowed to litter and rear the F₂ offspring to weaning. Dams were weighed on presumed gestation days 0, 3, 7, 10, 14, 17 and 20 then on days 0, 4, 7, 14 and 21 post partum. After birth, pups were weighed, individually identified by toe clip, sexed and weighed again on days 4, 8, 12, 16 and 21 post partum. Dams of the F₀ and F₁ generations were sacrificed by CO₂ asphyxiation at weaning of the pups on lactation day 21. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. All F₂ and the F₁ offspring not selected for the growth phase of the study were also sacrificed at weaning, weighed and evaluated at gross necropsy. Parameters observed in the F₁ generation during the pre-weaning period included: Surface Righting Reflex, Startle Reflex, Air Righting Reflex and Pupil Reflex. Parameters observed in the F₁ generation during maturation included date of vaginal opening in females and cleavage of the balanopreputial skinfold in males. Developmental and behavioral examinations included: Accelerating Rotarod Test, 'Actimat' Test, and Passive Avoidance Test. The following indices were calculated for the F₁ dams: Copulation, Fertility, Gestation, Birth, Day 4 Viability and Weaning Viability.

Results:**F₀ Generation:**

No maternal deaths were observed. No treatment-related clinical signs were observed in any group. At 4.9 mmol/kg/day maternal body weight gains were depressed and food consumption was minimally decreased during the treatment period only. Maternal effects on body weight and food consumption were not seen in the 0.1 and 0.7 mmol/kg/day groups.

F₁ and F₂ Generations:

Litter and fetal parameters were unaffected by treatment in the 0.1 and 0.7 mmol/kg/day groups. During the pre-weaning period, pups in the 0.7 mmol/kg/day exhibited a significant ($p < 0.05$) delay in attainment of the startle response and at 4.9 mmol/kg/day, startle response and air righting reflex were significantly ($p < 0.01$) delayed. During pregnancy of the F1 generation, body weight gain was reduced, but not significantly, for females of the 4.9 mmol/kg/day group. There were no other behavioral, external, visceral or skeletal effects observed.

SPONSOR'S CONCLUSION

The NOEL for maternal toxicity was 0.7 mmol/kg/day. The NOEL for fetal growth and development was 4.9 mmol/kg/day.

REVIEWER'S COMMENT

Disagree. The NOEL for maternal effects in this study is 0.7 mmol/kg/day. The NOEL for fetal liver lobation and sternbrae ossification effects is 0.7 mmol/kg/day.

**APPEARS THIS WAY
ON ORIGINAL**

**58. A Study Of The Effect of MP-1177/10 On Pregnancy Of The Rabbit. Study No. MLL 14/930473. In-
Life Study Dates, September 1-October 1, 1992. Report Dated March 20, 1995. Lot
Number S92120-C. In Compliance With GLP. Report In Volume 1.23, pp 287-378.**

Sponsor's Study 1101/05/92/024

Design: This study was designed to assess the maternal and fetal effects of MP-1177/10 (Optimark) when administered to pregnant rabbits on day 6-18 of presumed pregnancy. Sixty-four timed-pregnant New Zealand White rabbits weighing 3.1-4.0 kg and 16-24 weeks of age, were equally distributed among 4 treatment groups, and administered daily intravenous doses in the marginal ear vein of either saline or Optimark on days 6 through 18 of presumed gestation. A saline dosage of 3.2 mL/kg/day and Optimark dosages of 0.1, 0.4 and 1.6 mmol/kg/day were used. Dosages were equivalent to volumes of 3.2, 0.2, 0.8, and 3.2 mL/kg/day for Control through high dose, respectively, and were injected at a rate of 20 mL/minute. Dosages were selected based on a preliminary pilot study and a dose range finding study with doses ranging from 1.0-5.0 mmol/kg/day. Maternal lethality was produced at doses of 2.0 mmol/kg/day and above.

All rabbits were observed daily for survival and clinical signs. Body weights were recorded on Days 0, 2, 6, 8, 10, 14, 19, 23 and 29 of presumed gestation. Feed consumption values were recorded for the same intervals. Dams were sacrificed by cervical dislocation on day 29 of presumed gestation. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. The number and distribution of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded. All fetuses were weighed and evaluated for visceral and skeletal alterations and sex recorded. Indices for pre-implantation loss and post-implantation loss were calculated.

Results: No maternal deaths were observed. One dam in the Control group was excluded from the study prior to treatment due to poor physical condition. One dam in the Optimark 0.4 mmol/kg/day group spontaneously aborted. This single event is not considered related to treatment. No treatment-related clinical signs were observed in any group. There was some fluctuation in the maternal weight gain curves early in the treatment period, but these variations are considered spurious and not related to treatment. Food consumption was decreased in the 1.6 mmol/kg/day group during the treatment period only.

Litter and fetal parameters were unaffected by treatment. Soft tissue malformations observed in the 0.4 and 1.6 mmol/kg/day group included forelimb flexure and cardiovascular changes. The incidence of forelimb flexure was similar in this study to historical values seen in the conducting laboratory, however the incidence was lower for controls and the 0.1 mmol/kg/day group in this study than is normally seen in control groups at this laboratory. However, in the preliminary rabbit study, the number of fetuses with forelimb flexure was also increased at doses of 1.0 and 2.0 mmol/kg/day. Therefore, forelimb flexures are considered a treatment-related effect. The cardiovascular changes are also considered a result of Optimark treatment. No skeletal malformations or variations in the fetuses were considered effects of Optimark treatment.

STUDY SUMMARY TABLE

PARAMETER	TREATMENT GROUP			
	Control	0.1 mmol/kg	0.4 mmol/kg	1.6 mmol/kg
Dams Assigned	16	16	16	16
Dams Excluded	1			
Dams Treated	15	16	16	16
Aborted		1		
Not Pregnant	3	1	2	1
Live Litters	12	15	13	15
Mean Litter Corpora Lutea	10.6	11.3	10.5	11.1
Mean Litter Implants	9.3	9.7	9.0	9.5
Pre-Implant Loss (%)	13.5	15.0	15.6	13.6
Post-Implant Loss (%)	8.3	11.4	8.6	5.6
Total Group Live Pups	100	129	106	135
Mean Litter Live Pups	8.3	8.6	8.2	9.0
Mean Fetal Weight (g)	43.0	44.6	44.2	42.9
% Males	52.0	53.9	41.4	48.0
Malformed (fetuses/litters)	3/3	1/1	8/5	8/6
Forelimb Flexure	1	0	3	4
Cardiovascular Changes *	0	0	4	5

* Includes, malformed cervicothoracic and systemic/pulmonary arteries, ventricular septal defect, and enlarged or reduced ventricles.

SPONSOR'S CONCLUSION

The NOEL for maternal toxicity was 0.4 mmol/kg/day. The NOEL for fetal growth and development was 1.6 mmol/kg/day.

REVIEWER'S COMMENT

Disagree. The NOEL for maternal effects in this study is 0.4 mmol/kg/day. The NOEL for fetal cardiovascular effects and forelimb flexures is 0.1 mmol/kg/day. The NOEL for growth and other fetal effects is 1.6 mmol/kg/day.

**APPEARS THIS WAY
ON ORIGINAL**

60. A Study Of The Effect of MP-1177/10 On The Pregnant Rat And Offspring During The Peri- and Post Natal Period. Study No. MLL 18/930713.)

In-Life Study Dates, June 17-December 2, 1992. Report Dated January 5, 1995. Lot Number S92120-C. In Compliance With GLP. Report In Volume 1.24, pp 51-248.

Sponsor's Study 1101/05/92/025

Design: This study was designed to assess the maternal and fetal effects of MP-1177/10 (Optimark) when administered daily to pregnant rats from day 17 of presumed pregnancy through to day 21 post partum. One-hundred timed-pregnant CD⁰(SD) BR VAF/Plus] female rats weighing 200-220 g and 8-10 weeks of age, were equally distributed among 4 treatment groups, and administered daily intravenous doses in the tail vein, of either saline or Optimark. A saline dosage of 8.4 mL/kg/day and Optimark dosages of 0.1, 0.7 and 4.2 mmol/kg/day were used. Dosages were equivalent to volumes of 8.4, 0.2, 1.4, and 8.4 mL/kg/day for Control through high dose, respectively, and were injected at a rate of 1 mL/minute. Dosages were selected based on a preliminary dose range finding study with doses ranging from 1.0-4.9 mmol/kg/day, where retarded pup growth and minimal maternal weight depression were observed. All F₀ dams were allowed to give live birth and nurse the F₁ offspring to weaning at 21 days post partum. F₀ dams were the only generation treated, and were treated only from day 17 of presumed gestation through to day 21 post partum. All dams were observed daily for survival and clinical signs. Body weights were recorded on Days 0, 2, 4, 7, 8, 10, 12, 14, 18 and 20 of presumed gestation. Feed consumption values were recorded for the same intervals. Dams were allowed to deliver live litters and nurse the offspring to weaning. The following indices were calculated for the F₀ dams: Gestation, Birth, Day 4 Viability and Weaning Viability.

F₁ and F₂ Generations:

Randomly selected F₁ offspring of 10/sex/ group were retained to maturity, paired (not with litter mates) and allowed to litter and rear the F₂ offspring to weaning. Dams were weighed on presumed gestation days 0, 3, 7, 10, 14, 17 and 20 then on days 0, 4, 7, 14 and 21 post partum. After birth, pups were weighed, individually identified by toe clip, sexed and weighed again on days 4, 8, 12, 16 and 21 post partum. Dams of the F₀ and F₁ generations were sacrificed by CO₂ asphyxiation at weaning of the pups on lactation day 21. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. All F₂ and the F₁ offspring not selected for the growth phase of the study were also sacrificed at weaning, weighed and evaluated at gross necropsy. Parameters observed in the F₁ generation during the pre-weaning period included: Surface Righting Reflex, Startle Reflex, Air Righting Reflex and Pupil Reflex. Parameters observed in the F₁ generation during maturation included date of vaginal opening in females and cleavage of the balanopreputial skinfold in males. Developmental and behavioral examinations included: Accelerating Rotarod Test, 'Actimat' Test, and Passive Avoidance Test. The following indices were calculated for the F₁ dams: Copulation, Fertility, Gestation, Birth, Day 4 Viability and Weaning Viability.

Results:**F₀ Generation:**

No maternal deaths were observed. No treatment-related clinical signs were observed in any group. The only treatment related effects seen in the F₀ dams occurred in the 4.2 mmol/kg/day group, where an increase in the incidence of a red-brown peri-orbital discharge was noted in most of the females during the third week of lactation. Maternal effects on body weight and food consumption were not seen in any of the groups. There were no deleterious effects related to treatment in the 0.1 and 0.7 mmol/kg/day on the F₀ dams or their untreated F₁ offspring.

F₁ and F₂ Generations:

F₁ litter and fetal parameters were unaffected by treatment in the 0.1 and 0.7 mmol/kg/day groups. In the 4.2 mmol/kg/day F₁ group, mean implantation loss, reflecting pup losses in utero, was higher than controls which was exhibited by a reduction of litter size at birth. These differences were not statistically significant at ($p \leq 0.05$). This effect was a result of 2 of 25 litters with high implantation losses of 69.2% and 40.0%. At 4.2 mmol/kg/day, F₁ litter weight at birth and through to weaning was reduced, compared to controls, with statistical significance ($p \leq 0.05$) continuing from lactation day 8 through to weaning. One moribund F₁ generation dam, in the 0.1 mmol/kg/day group, along with her F₂ generation litter were sacrificed on day 9 post partum. The dam was found to have an ulcerated subcutaneous mass in the thoracic region, and was considered incidental and not related to treatment. During the post-weaning period, F₁ male pups in the 0.7 mmol/kg/day and 4.9 mmol/kg/day groups exhibited a significant ($p \leq 0.05$) increase in mean maximum performance times in the Rotarod Test. There were no other behavioral, external, visceral or skeletal effects observed. No effects were observed for any groups of F₂ fetuses and pups.

SPONSOR'S CONCLUSION

The NOEL for maternal toxicity was 0.7 mmol/kg/day. The NOEL for fetal growth and development was 4.2 mmol/kg/day.

REVIEWER'S COMMENT

Disagree. The NOEL for maternal effects in this study, based on periorbital discharge during the third week of lactation is 0.7 mmol/kg/day. The NOEL for reduced weight at birth through weaning is also 0.7 mmol/kg/day.

The F₁ animals were exposed in utero approximately 5 days and exposed via milk through weaning. This would suggest that drug was absorbed systemically or interfered with nutrition in some way by the drug's presence in milk. Systemic exposure via milk was not assessed in this or other reprotoxicity or PK studies.

Summary of Reproductive Toxicology-by John Melograna

Seven reproductive toxicology studies were submitted to this NDA; 3 were pilot investigations; 4 were definitive studies which were reviewed in detail:

- a fertility and reproductive performance study in rats
- 2 pregnancy studies: 1 in rats, 1 in rabbits
- a peri- and post-natal study in rats

The first study, the fertility and reproductive performance study in rats, included investigation of effects on male and female fertility (F_0) and on 2 generations of offspring (F_1 and F_2). In this study, doses of 0, 0.1, 0.5, and 2.0 mmole/kg MP-1177/10 were administered iv to male rats for 9 weeks plus a 20 day cohabitation period, and to female rats from 2 weeks before mating through Day 20 of gestation. Thus F_1 pups were not exposed via milk during lactation. Since males in the high dose group displayed signs of severe toxicity, their dose regimens were terminated after 7 weeks and they were tested separately for fertility by pairing with untreated females after 4 and 8 week recovery periods. F_1 and F_2 offspring were not treated.

Doses of 0.1 and 0.5 mmole/kg did not produce toxic effects in F_0 males and females as measured by mortality, clinical signs, body weight, and food consumption. Neither were fertility effects found at these doses.

The high dose of 2.0 mmole/kg to males for 7 weeks significantly reduced body weight and food consumption and produced toxicity to the male reproductive system as follows: histopathological findings of irreversible reduction and degeneration of spermatocytes in the testes and epididymides and reduced fertility 4 and 8 weeks after termination of dosing. Actual fertility results at 2.0 mmole/kg were: 1/12 pregnancies for 11/12 matings at 4 weeks and 1/12 pregnancies for 2/12 matings at 8 weeks. Overall NOAEL for F_0 males=0.5 mmole/kg.

Body weights of F_1 offspring (from F_0 females at 0.5 mmole/kg) at birth through weaning were reduced (NOAEL=0.1 mmole/kg). No other effects were found in F_1 (Caesarean and littered) and F_2 offspring as measured by litter and fetal parameters; external, visceral and skeletal exams; markers of development (reflexes and sexual maturity); behavioral tests, and indices of reproduction (F_1 only).

The second study, a developmental toxicity study in rats, included investigation of MP-1177/10 effects on pregnant rats and 2 generations of offspring. Doses of 0, 0.1, 0.7, and 4.9 mmole/kg were administered iv to pregnant rats on days 7-17 of gestation. F_1 and F_2 offspring were not treated.

Doses of 0.1 and 0.7 mmole/kg did not produce toxic effects in F_0 females as measured by mortality, clinical signs, body weight, and food consumption. The high dose, 4.9

mmole/kg, produced significant depression of body weight gain and a slight decrease in food consumption.

F₁ pups delivered by Caesarean section were not affected by maternal doses of 0.1 and 0.7 mmole/kg as measured by litter and fetal parameters and visceral and skeletal exams. F₁ fetal effects at 4.9 mmole/kg were retarded growth, abnormal liver lobation, and delayed ossification of the sternebrae.

F₁ pups raised to maturity were not affected by the low dose, 0.1 mmole/kg, as measured by markers of development (reflexes and sexual maturity), behavioral tests, and indices of reproduction. However, the development of the startle response was delayed in 0.7 and 4.9 mmole/kg offspring, and air righting reflex was delayed in 4.9 mmole/kg offspring. During pregnancy of the F₁ females from the 4.9 mmole/kg group, body weight was slightly reduced.

No effects were found in the F₂ generation.

In the third study conducted in pregnant rabbits, doses of 0, 0.1, 0.4 and 1.6 mmole/kg were administered iv to pregnant rabbits on gestation days 6 through 18. Maternal and fetal effects of MP-1177/10 were evaluated.

F₀ females receiving 0.1 and 0.4 mmole/kg were not affected by treatment as measured by mortality, clinical signs, body weight and food consumption. The high dose, 1.6 mmole/kg, produced decreased food consumption during the treatment period only.

F₁ fetuses were evaluated for litter and fetal parameters and visceral and skeletal defects. Cardiovascular changes were found at 0.4 and 1.6 mmole/kg: malformed thoracic arteries, 1 septal defect, and 1 case of abnormal ventricle. Forelimb flexures were also found at 0.4 and 1.6 mmole/kg. Although incidences were within historical controls for the conducting laboratory, the controls from this study had a lower incidence than treated groups, and a preliminary study gave similar results. Therefore, cardiovascular changes and forelimb flexures are both considered treatment-related effects.

In the fourth study, effects of MP-1177/10 on rats during pregnancy (F₀ and F₁) and on offspring during the peri- and postnatal periods (F₁ and F₂) were evaluated. Doses of 0, 0.1, 0.7 and 4.2 mmole/kg were administered iv to F₀ rats from day 17 of gestation through day 21 post partum. All pregnant rats were allowed to litter and nurse their offspring. F₁ and F₂ pups were not directly treated.

F₀ females were unaffected by treatment with 0.1 and 0.7 mmole/kg as measured by mortality, clinical signs, body weights, food consumption, indices of reproductive performance, and litter data (for F₀ females delivering F₁ offspring). F₀ females treated with 4.2 mmole/kg secreted a red-brown peri-orbital discharge during the 3rd week of lactation. Also, 2 of 25 litters in the 4.2 mmole/kg group had high implantation losses

(69% and 40%); however, the Sponsor pointed out that implantation occurred prior to commencement of dosing (day 17 of gestation).

Adverse effects as measured by growth (bw), markers of development (reflexes and sexual maturity), behavioral tests, and as adults, indices of reproductive performance were not detected.

During the post-weaning period, male pups in the 0.7 and 4.2 mmole/kg groups exhibited significant increases in mean maximum performance times in the Rotarod test. However, this is not considered an adverse effect.

Litter data for F₁ rats delivering F₂ offspring were not affected by the F₀ treatment group they came from.

Summary tables of NOAELs for reproductive toxicity study effects follows:

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NOAELS For Reproductive Toxicity Study Effects of MP-1177/10				
Study number/type	Doses (mmole/kg)	Effects	NOAEL	
			mmole/kg	multiple of human dose*
54. Fertility And Reproductive Performance In Rats	0	F ₀ males-reduced body weight and food consumption, toxicity to the male reproductive system including irreversible reduction and degeneration of spermatocytes in testes and epididymides, and reduced male fertility, vacuolization of the proximal tubular epithelium of the kidneys F ₀ females-all measures (note females not treated at high dose) F ₁ fetuses and pups-reduced body weight birth through weaning -all other measures F ₂ pups-all measures	0.5	1
	0.1			
	0.5			
	2.0			
55. Developmental Toxicity Study In Rats	0	F ₀ females-decreased body weight and food consumption -all other measures F ₁ fetuses-retarded growth, abnormal liver lobation, and delayed ossification of sternbrae -all other measures F ₁ pups-delayed development of startle response -delayed development of air righting reflex -decreased body weight in females during pregnancy -all other measures F ₂ pups-all measures	0.7	1
	0.1			
	0.7			
	4.9			
*NOAEL as multiple of human dose of 0.1 mmole/kg based on body surface area comparison				

NOAELS For Reproductive Toxicity Study Effects of MP-1177/10 (continued)				
Study number/type	Doses (mmole/kg)	Effects	NOAEL	
			mmole/kg	multiple of human dose*
58. Developmental Toxicity In Rabbits	0	F ₀ females-decreased food consumption during treatment period only -all other measures	0.4	1
	0.1		>1.6	>5
	0.4	F ₁ fetuses-cardiovascular changes (malformed thoracic arteries, septal defect, and abnormal ventricle) -forelimb flexures -all other measures	0.1	0.3
	1.6		0.1	0.3
		>1.6	>5	
60. Peri- and Post-Natal Study In Rats	0	F ₀ females-red-brown peri-orbital discharge during 3 rd week of lactation -implantation losses of 69% and 40% in 2/25 litters at 4.2 mmole/kg (not treatment related) -all other measures	0.7	1
	0.1		0.7	1
	0.7		>4.2	>7
	4.2	F ₁ pups-all measures	>4.2	>7

*NOAEL as multiple of human dose of 0.1 mmole/kg based on body surface area comparison

Genotoxicology

61. Title: Salmonella / Mammalian Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and Escherichia Coli WP2 UVRA Reverse Mutation Assay with a Confirmatory Assay

Study#: 1101/05/92/012

Test System: Salmonella strains TA98, TA100, TA1535, TA1537, TA1538 and E.coli strain WP2uvrA

Test Conditions: with and without Aroclor-induced rat liver microsomal enzymes

Formulation: MP-1177/10

Controls:

Strain	Activation	Positive Control	Concentration (µg/plate)
TA98	+	2-aminoanthracene	1.0
TA98	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	1.0
TA100	-	sodium azide	1.0
TA1535	+	2-aminoanthracene	1.0
TA1535	-	sodium azide	1.0
TA1537	+	2-aminoanthracene	1.0
TA1537	-	9-aminoacridine	75
TA1538	+	2-aminoanthracene	1.0
TA1538	-	2-nitrofluorene	1.0
WP2 uvrA	+	2-aminoanthracene	10,000
WP2 uvrA	-	methyl methanesulfonate	1,000

Dose Range: 100 to 5,000 ug/plate

Study Design: dose ranging study, mutagenicity assay, confirmatory assay

Conformance with current ICH guidelines:

- 1) tester strains: adequate
- 2) top dose: cytotoxicity was not observed at the high dose, the highest dose was 5 mg/plate which was appropriate for a nontoxic compound.
- 3) solubility: precipitate was not observed at any dose

Results: negative

62. Title: L5178Y TK +/- Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay

Study#: 1101/05/92/013

Test System: cultured L5178Y mouse lymphoma cells

Test Conditions: with and without Aroclor induced rat liver S-9

Formulation: MP-1177/10

Controls: solvent, 7,12-dimethylbenz(a)anthracene ("with activation" positive control), ethyl methanesulfonate ("without activation" positive control)

Study Design and Dose Ranges:

toxicity test 0.5 to 5,017 ug/ml
 mutagenicity assay 1750 to 5,017 ug/ml
 confirmatory assay 1500 to 5,017 ug/ml

Conformance with current ICH guidelines:

1) **top concentration:** the highest dose concentration was 5.017 mg/ml which is appropriate for a non-toxic compound with MW>500 g/mole (MW gadoversetamide=662 g/mole)

2) **solubility:** not mentioned, but expected to be freely soluble

Results: negative

63. Title: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells

Study#: 1101/05/92/014

Test System: cultured Chinese hamster ovary cells

Test Conditions: with and without Aroclor-induced rat liver S-9

Formulation: MP-1177/10

Controls: non-activated positive control-N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)
 activated positive control-benzo(α)pyrene (B[α]P)

Study Design and dose ranges:

preliminary toxicity assay 0.5-5,000 ug/ml

definitive assay 630-5,000 ug/ml

confirmatory assay 630-5,000 ug/ml

Conformance with current ICH guidelines:

1) **Top concentration:** the highest dose concentration, 5 mg/ml, did produce some toxicity at 24 and 48 hr in non-activated cultures

Survival at high concentration relative to control		
	non-activated	activated
6 hr	95%	101%
24 hr	79%	not tested
48 hr	41%	not tested

Results: positive in test condition without activated S-9

negative in test condition with activated S-9

Sponsor's Conclusion:

MP-1177/10 induced a statistically significant and reproducible increase in structural chromosome aberrations at 5000 µg/ml in the nonactivated 24 hour treatment condition and in numerical chromosome aberrations at 5000 µg/ml in the 48 hour treatment condition. Statistically significant increases in structural or numerical chromosome aberrations at other dose levels and treatment conditions were not reproducible although some of these increases were clearly dose related (i.e., 48 hour treatment condition, confirmatory study). Based upon the findings of the initial and the confirmatory studies, MP-1177/10 was concluded to be weakly positive in the chromosome aberration assay using Chinese hamster ovary cells.

64. Title: Micronucleus Cytogenetic Assay in Mice**Study#:** 1101/05/92/015**Test System:** live ICR mice**Formulation:** MP-1177/10**Controls:** positive-cyclophosphamide (CP), negative-vehicle**Doses:** 1250, 2500, and 5000 mg/kg (1.9, 3.8, 7.6 mmole/kg respectively)**Study Design:** Pilot study-mortality studied in mice receiving iv doses of MP-1177/10

Micronucleus assay-13 groups of 5 males and 5 females per group were dosed by the iv route with vehicle, MP-1177/10, or CP and terminated as follows:

	Animals per Sex Sacrificed After Dose Administration		
	<u>24 hr</u>	<u>48 hr</u>	<u>72 hr</u>
Vehicle Control	5	5	5
Low test dose (1250 mg/kg)	5	5	5
Mid test dose (2500 mg/kg)	5	5	5
High test dose (5000 mg/kg)	5	5	5
CP, 30 mg/kg	5		

Results: negative**Sponsor's conclusion:**

No reduction in the ratio of polychromatic erythrocytes to total erythrocytes was observed in the test article-treated groups relative to the vehicle control suggesting that the test article did not induce bone marrow toxicity. No significant increases in micronucleated polychromatic erythrocytes were observed at 24, 48 or 72 hours after dose administration in males or females. The results of the assay indicate that under the conditions described in this report, MP-1177/10 did not induce a significant increase in micronucleated polychromatic erythrocytes in either male or female ICR mice. MP-1177/10 was concluded to be negative in the mouse micronucleus assay.

Reviewer comments:

The doses in this study are 1.6, 3.2, and 6.3 times the human dose based on a body surface area comparison. The high dose in this study (7.6 mmole/kg) produced 2 lethalties. The maximum non-lethal dose found in single dose mouse (CD-1 and ICR) studies discussed elsewhere in this review ranged from 10-14 mmole/kg.

Summary of Genotoxicology

The Sponsor has conducted an adequate array of genotoxicity studies by FDA standards (see ICH S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals). According to this standard, the battery should consist of at least 3 tests. These recommended tests are listed below with the studies the Sponsor conducted to comply with the recommendations:

ICH S2B Recommended Test	Test Conducted by Sponsor which fulfills ICH S2B Recommendation
A test for gene mutation in bacteria	Salmonella / Mammalian Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and Escherichia Coli WP2 UVRA Reverse Mutation Assay with a Confirmatory Assay
An in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells or an in vitro mouse lymphoma tk assay	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells and L5178Y TK +/- Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay
An in vivo test for chromosomal damage using rodent hematopoietic cells	Micronucleus Cytogenetic Assay in Mice

All tests were negative except the Chromosome Aberrations in CHO cells assay. This assay was positive at the highest concentration 5000 ug/ml (7.6 umole/ml) in cultures not activated with S-9.

The positive result should be reported in the labeling.

Special Toxicology

The first 3 studies in the Special Toxicity section are local irritation studies which are summarized in the following table.

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Summary of Local Irritation Studies

Study	Species	Design	Procedure	Results
65. Venous Irritation of MP-1177/10 Injection in Rabbits (Study 1101/05/93/011)	rabbit	1. MP-1177/10 (n=6♀s) 2. bromosulfonthalein (BSP) pos cntrl (n=6♀s) saline negative control (n=12) – contralateral ear in groups 1 & 2	0.05 ml of 0.5 M MP-1177/10 or positive control injected twice daily for 8 days into clamped 3 cm section of right retroauricular vein for 3 min before clamp removed. Same procedure for saline in contralateral vein	macroscopic-no difference from saline controls microscopic-irritation slightly greater than saline controls (mild phlebitis/peri-phlebitis) 1 animal with mild necrosis, 1 with perivascular neofibrosis
66. Comparative Study of the Venous Irritation of MP-1177/10 Injection and Magnevist® in Rabbits (Study 1101/05/93/016)	rabbit	1. MP-1177/10 (n=3♀s) 2. Magnevist (n=3♀s) 3. bromosulfonthalein (BSP) pos cntrl (n=3♀s) saline negative control (n=9) – contralateral ear in groups 1, 2 & 3	same as Study 65.	macroscopic-no difference between Magnevist, MP-1177/10 and saline control microscopic- irritation slightly greater than saline controls except 1 animal with thrombosis and necrosis comparable to positive control. MP-1177/10 appeared slightly more irritating than Magnevist but number of animals considered too low to make conclusions
67. Intramuscular and subcutaneous Irritation of MP-1177/10 in Rabbits (Study 1101/05/92/029)	rabbit	24 animals, n=4/group or 2♂s & 2♀s/group received both an im and sc injection once and then sacrificed 2 or 14 days later as specified 1. MP-1177/10-2 days 2. MP-1177/10-14 days 3. 0.45% acetic acid-2 days 4. 0.45% acetic acid-14 days 5. 1.7% acetic acid-2 days 6. 1.7% acetic acid-14 days saline negative control (n=24)- injected into both contralateral sites	1.0 ml of 0.5 M MP-1177/10 or positive control was injected 1) im over 10 sec at depth 1 cm into vastus lateralis and 2) sc into anterior surface of radius of same rabbits. Same procedure for saline in both contralateral sites of each rabbit.	macroscopic-no difference from saline controls by either route microscopic- no difference from saline control by either route

Conclusions of Local Irritation Studies:

Subcutaneous and intramuscular administration of 1.0 ml of a standard concentration (0.5 M) of MP-1177/10 did not produce irritation in rabbits.

However, multiple exposures of MP-1177/10 intravenously into a clamped section of vein produced irritation slightly greater than saline controls in most animals, and greater irritation in a few of the animals as follows: mild necrosis (1/6), perivascular neofibrosis (1/6), thrombosis and necrosis comparable to positive control (1/3).

68. Title: In vitro Human Blood Compatibility of MP-1177/10 (Study 1101/05/92/019)

Synopsis:

Solution	Purpose	Parameter	Mean Result
0.9% sodium chloride	negative control	hemolysis RBC crenation plasma precipitation	no data 4% negative
0.1% acetic acid	positive control	hemolysis	no data
*40% EBSS	positive control	RBC crenation	79%
0.5 M MP-1177/10	experimental	hemolysis RBC crenation plasma precipitation aggregation	1% 5% negative negative

*EBSS=Earls' Balanced-Salt Solution-10X

Conclusion:

These data support that MP-1177/10 is compatible with human blood.

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69. Title: Comparative Blood and Plasma Compatibility of MP-1177/10 Injection and Magnevist® (Study 1101/05/93/022)

Synopsis:

Experiment	Solution	Purpose	Parameter	Mean Result
1	0.9% saline	negative control	hemolysis	set at 0%
	0.1% acetic acid	positive control		set at 100%
	0.5 M MP-1177/10	experimental		0.19%
	Magnevist	experimental		0.41%
2	0.9% saline	negative control	RBC crenation	0%
	*40% EBSS	positive control		49%
	0.5 M MP-1177/10	experimental		1.7%
	Magnevist	experimental		0.3%
3	0.9% saline	negative control	protein precipitation	20 mg/ml
	0.5 M MP-1177/10	experimental		24 mg/ml
	Magnevist	experimental		21 mg/ml

*EBSS=Earls' Balanced Salt Solution 10X in DuBecco's Modified Phosphate Buffered Saline Solution

Conclusion:

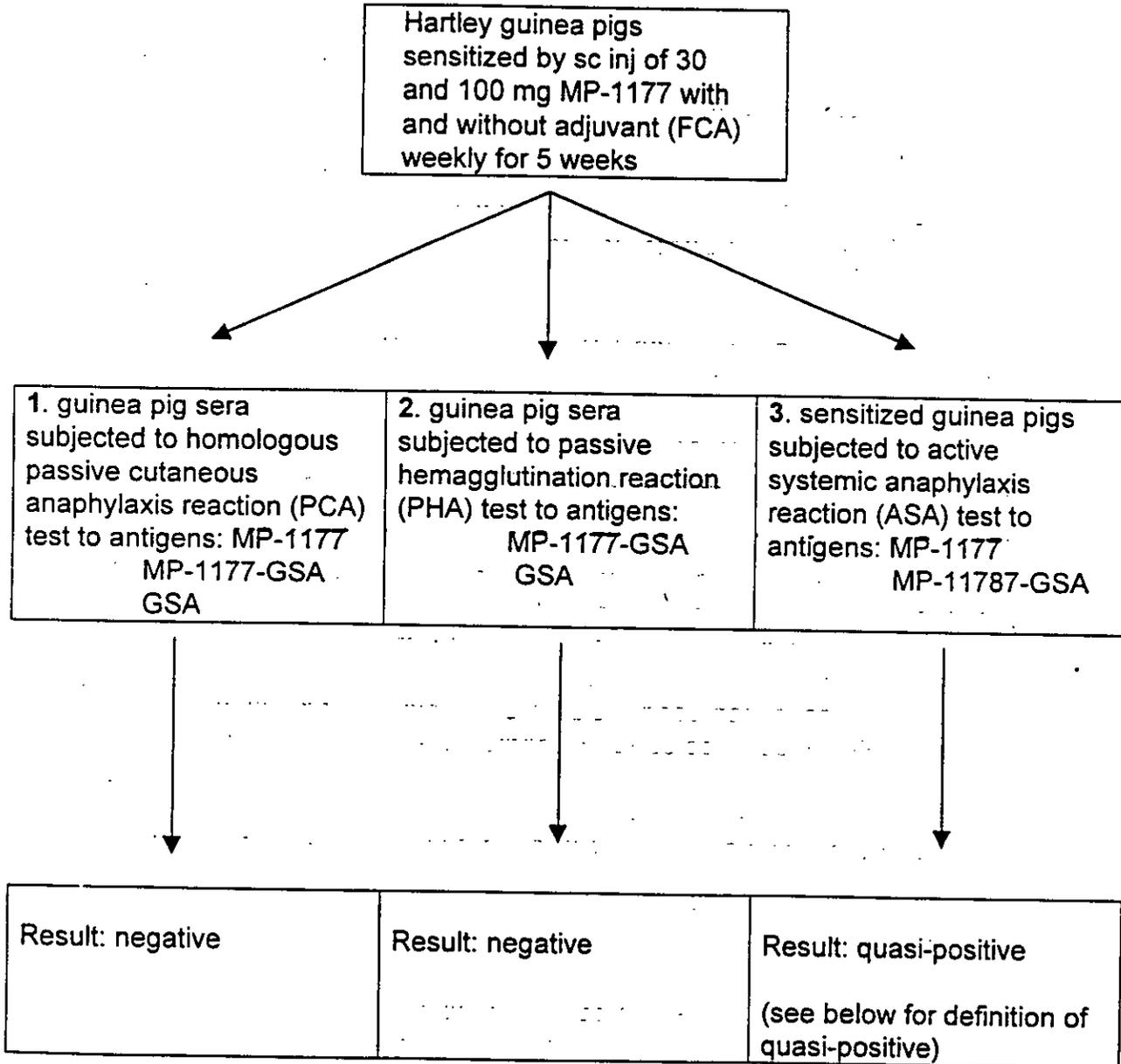
These data support that MP-1177/10 is compatible with human blood and that Optimark and Magnevist are equivalent in terms of blood computability.

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70. Title: Antigenicity Testing of MP-1177

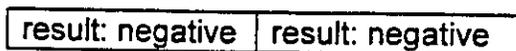
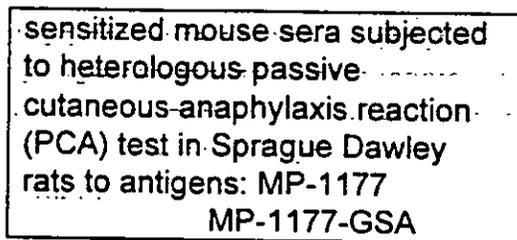
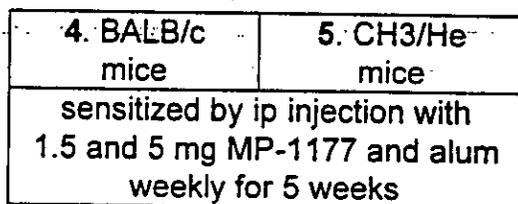
Synopsis:

Five antigenicity tests were conducted in this study. Three of the tests were conducted in Guinea pigs as follows:



The definition of quasi-positive is one of the ratings of the ASA test listed below:

Signs	Evaluation
No observable signs or spurious symptoms resembling systemic anaphylactic reaction (e.g. preening, normal urination and evacuation etc.)	: - (negative)
Sparse reactions such as rubbing or licking the nose and ruffling the fur	: ± (quasi-positive)
Frequent appearance of the above mentioned symptoms and development of sneeze, cough, stridor, weakness or restlessness	: + (positive)
Development of retching, evacuation, urination (incontinence), dyspnea, convulsion or prostration in addition to the above mentioned symptoms	: ++ (strongly positive)
Death (until 24 hr after administration)	: +++ (severely positive)



Conclusion: In this screen of 5 tests, MP-1177 was very weakly antigenic.

71. Title: Primary Screening of a Test Article for Antagonism to Histamine Using the Isolated Guinea Pig Trachea (Pilot Study) (Study 1101/05/93/027-E)

Synopsis: This study was conducted to determine the effects of the Optimark formulation, MP-1177/10, and Magnevist on histamine induced contraction of an isolated guinea pig trachea preparation.

A single concentration of 15 mM of each gadolinium agent was tested. (To put the concentration into perspective: note that the theoretical maximum plasma concentration following a 0.1 mmol/kg dose is 2.5 mM, assuming instantaneous distribution to the plasma compartment.) The 15 mM concentration of MP-1177/10 and Magnevist reduced contraction by 49% and 78% respectively. The respective controls (0.9% saline) reduced contraction by 9% and 5%.

Reviewer comment: Both test articles produced relaxation of histamine induced contraction of the isolated guinea pig trachea. Lower concentrations were tested in the next study.

72. Title: Primary Screening of MP-1177/10 and Magnevist® for Effects Upon Histamine or Acetylcholine Induced Contraction Using the Isolated Guinea Pig Trachea (Study 1101/05/93/028-E)

Synopsis: This study was conducted to determine the effects of different concentrations of the Optimark formulation, MP-1177/10, and Magnevist on histamine-induced and acetyl choline induced contractions of an isolated guinea pig trachea preparation.

Three concentrations of each gadolinium agent were tested: 1.5, 5.0 and 15 mM. (To put the concentration into perspective, note that the theoretical maximum plasma concentration following a 0.1 mmol/kg dose, and assuming instantaneous distribution to the plasma compartment, is 2.5 mM.) Results are presented in the following table:

Effect of MP-1177/10 and Magnevist on Histamine- and Acetylcholine-induced contraction of the Isolated Guinea Pig Trachea			
Test Solution	Volume or Concentration	Percent reduction in trachea contraction	
		Histamine-induced (2 μ M)	Acetylcholine-induced (5 μ M)
Saline	90 μ l	4	4
	300 μ l	7	7
	900 μ l	6	9
MP-1177/10	1.5 mM	9	7
	5.0 mM	13	27
	15 mM	45	44
Saline	90 μ l	1	5
	300 μ l	6	4
	900 μ l	7	8
Magnevist	1.5 mM	7	7
	5.0 mM	19	16
	15 mM	46	36

Synopsis: Both test articles produced concentration-related relaxation of histamine- and acetylcholine-induced contractions of the isolated guinea pig trachea. Since the responses were similar in the histamine and acetylcholine systems; it is suggested that MP-1177/10 and Magnevist do not inhibit smooth muscle contraction by receptor antagonism.

This response would not be expected at the proposed human dose level of 0.1 mmole/kg.

73. **Title:** Neurotoxicity of Extracellular MRI Agents

Study #: 1101/05/93/031

Species/Strain/Source: rats/Sprague-Dawley

Sex/age/body weight: female/6 weeks

Dose information:

Formulation: MP-1177/10 and Magnevist (marketed formulation), controls received saline only

Concentration(s): 0.5 M

Dosages: 0, 50 and 100 μ mole/kg of both agents

Route of administration: intracisternal

Volume of administration: 200, 100, and 200 μ l/kg respectively

Rate of administration: not given

Study design and schedule:

Group ID	MP-1177/10 Injection Dose ($\mu\text{mol/kg}$)	Magnevist ^a Dose ($\mu\text{mol/kg}$)	Dose Volume ($\mu\text{L/kg}$)	Number of Female Rats
Va	0	0	200	12
A	50	0	100	6
B	100	0	200	6
C	0	50	100	6
D	0	100	200	6

^aThese animals were dosed with saline only.

Note that doses of 50 and 100 $\mu\text{mol/kg}$ by the intracisternal route correspond to estimated concentrations of in the CSF at least 20 and 40 times C_{max} in humans following an iv dose. C_{max} is a crude estimate of the maximum possible concentration in brain of humans with a disrupted blood brain barrier. See study #42 of this review for a more detailed explanation.

PROCEDURE	TIME OF PROCEDURE /DATA COLLECTION
acclimation, Rotarod training, and deselection of animals not performing well	during 2 weeks prior to dosing
dose administration under metofane anesthesia	time=0
clinical observations for signs of toxicity	1,2 and 4 hr after dosing and daily for 6 days
mortality	twice daily
Rotarod evaluations	following clin obs at 1,2 and 4 hr after dosing and daily for 6 days
body weights	daily
whole body perfusion with Karnovsky's fixative under anesthesia	6 days after dosing
histopathological work-up of brain, spinal cord, spinal nerve roots, and dorsal root ganglia (tissues dissected, processed, embedded in parafin, sectioned and stained with hematoxylin and eosin)	following whole body perfusion

Results:

Mortality: none

Clinical signs:

Incidence of Clinical Signs Observed in Intracisternal Neurotoxicity Study (Days* that signs appeared in parentheses)					
	vehicle	MP-1177/10		Magnevist	
		50 umole/kg	100 umole/kg	50 umole/kg	100 umole/kg
tremors	5/12 (D1)	5/6 (D1)	3/6 (D1)	3/6 (D3)	3/6 (D3)
ataxia	4/12 (D1)	1/6 (D1)	1/6 (D1)	6/6 (D1,3,4)	6/6 (D1,3,4)
hypoactivity	2/12 (D1)	3/6 (D2,3,4)	4/6 (D2,3,4)	4/6 (D1,3,4)	4/6 (D1,3,4)
hyperexcitability	2/12 (D1)	1/6 (D1)	0/6	0/6	0/6
tilted head	1/12 (D2-7)	0/6	0/6	0/6	0/6
tense body	0/12	2/6 (D1)	5/6 (D1)	3/6 (D1,2)	3/6 (D1,2)
lethargy	0/12	0/6	0/6	4/6 (D1)	4/6 (D1)
normal by day	2	5	5	5	5

*In this study, Day 1 was the day of dosing, Day 2 was the day after dosing....Day 7 was 6 days after the day of dosing.

Body weight: Body weight gain was slowed in the 100 umole/kg Magnevist group. All other treatment groups were similar to controls.

Rotarod performance:

Mean Rotarod Time (minutes)									
	1 hr	2 hr	4 hr	day 2	day 3	day 4	day 5	day 6	day 7
saline	1.63	1.91	1.94	1.93	1.85	2.00	2.00	1.94	2.00
50 umole/kg MP-1177/10	2.00	2.00	1.90	1.71	1.78	1.76	1.86	1.71	2.00
100 umole/kg MP-1177/10	1.72	1.76	1.89	2.00	1.89	1.76	2.00	2.00	1.74
50 umole/kg Magnevist	1.07	1.76	2.00	2.00	2.00	2.00	2.00	2.00	2.00
100 umole/kg Magnevist	0.37	1.33	1.74	1.62	1.42	2.00	1.89	1.90	1.98

Neuropathology: No effect

Reviewer comments: MP-1177/10 administration did not affect body weights or rotarod performance nor did it produce histopathological effects on brain, spinal cord, spinal nerve roots or dorsal root ganglia. Effects on clinical signs were minimal and reversible. Magnevist produced minimal and reversible effects on body weight, rotarod performance, and clinical signs but not histopathological effects.

Summary of Special Toxicology

Special toxicology studies included local irritation studies, human blood compatibility studies, antigenicity studies, and an intracisternal neurotoxicity study.

Local irritation studies included venous irritation, intramuscular and subcutaneous irritation studies, all conducted in rabbits. The venous irritation studies were conducted under exaggerated conditions: 0.5 M MP-1177/10 was injected into a clamped section of the vein and kept there for 3 min; this was done 16 times over 3 days. Under these conditions treated animals showed a mild reaction (mild phlebitis and periphlebitis). However, mild necrosis and perivascular necrosis was also observed in 1 of 3 animals in one study and thrombosis and necrosis comparable to the positive control was found in 1 of 3 animals from the other study. The Sponsor concluded that MP-1177/10 was mildly irritating to veins. In the reviewers opinion, it is more than mildly irritating under the conditions of this study. It would be useful to know if the more significant effects could have been avoided by reducing the number of doses.

Results of intramuscular and subcutaneous irritation studies were negative when the equivalent of 0.2 mmole/kg was injected into im and sc sites.

Data from human blood compatibility studies support that MP-1177/10 is compatible with human blood.

In a screen of antigenicity tests, it was concluded that MP-1177/10 was very weakly antigenic. This was based on results of a guinea pig active systemic anaphylaxis (ASA) assay in which "sparse reactions such as rubbing or licking the nose and ruffling the fur" were observed. Negative assays included homologous passive cutaneous anaphylaxis (PCA) and passive hemagglutination (PHA) assays in guinea pigs, and heterologous PCA assays (serum from 2 strains of sensitized mice tested in rats).

MP-1177/10 produced concentration-related relaxation of histamine- and acetylcholine-induced contractions of the isolated guinea pig trachea. Since the responses were similar in the histamine and acetylcholine systems, it is concluded that MP-1177/10 does not inhibit smooth muscle contraction by antagonism of these receptors. The

effect of MP-1177/10 was similar to Magnevist in this assay. This effect would not be expected at the proposed human dose level of 0.1 mmole/kg.

In an intracisternal neurotoxicity study in rats, MP-1177/10 administration did not affect body weights or rotarod performance nor did it produce histopathological effects on brain, spinal cord, spinal nerve roots or dorsal root ganglia. The high dose in this study, 100 umole/kg, was estimated to produce a CSF concentration at least 40 times the estimated brain concentration in patients with disrupted blood:brain barrier.

Overall Summary

NDA 20-937 has been submitted to apply for approval to market Optimark for MRI contrast enhancement of liver lesions, lesions of the spine and associated tissues, and intracranial lesions with abnormal vascularity or abnormal blood brain barrier. The Sponsor submitted 73 non-clinical animal studies to characterize the safety of administration of Optimark. Note that the formulation, MP-1177/10, which was tested in most animal studies is the same as the Optimark formulation. MP-1177 is gadoversetamide. MP-1196 is versetamide.

Pharmacokinetic studies were conducted in rats and dogs and compared to human data. MP-1177/10 was similar across species when administered at 0.1 mmole/kg as follows: 1) Following iv injection, MP-1177/10 distributed to the extracellular fluid volume, 2) elimination was almost solely by the urinary route, 3) elimination was rapid (about 80-95% within 4 hours), 4) biotransformation was not detected in biological fluids, and 5) protein binding was not detected.

MP-1177/10 is eliminated more rapidly in laboratory animals than in humans: 1) rat and dog late phase half-lives were 6-fold and 2-fold lower than for humans respectively, and 2) plasma clearance rates were 8-fold and 3-fold greater than for human respectively, and 3) rat and dog plasma AUC values were about 6-fold and 2.5-fold lower respectively than the mean human serum AUC. These data support that dose comparisons between animals and humans in toxicology studies based on body surface area are appropriate.

Animal data demonstrated less than 5% of radioactivity in feces following administration of [^{153}Gd] MP-1177/10. In experimentally induced anephria in the rat, hepatobiliary excretion did not compensate for blockage of urinary elimination. Therefore, dialysis may be needed to clear Optimark if it is administered to a renally impaired subject.

Biodegradation of MP-1177/10 in anephria was not tested but it is a concern. In rats with normal kidney function, small levels of gadolinium (0.2 to 0.5% inj dose/organ) persist in liver and kidney 24 to 48 hr post administration in rats. By 7 days, these levels decreased (to 0.1-0.2% inj dose/organ) while bone levels increased (to 0.3% inj dose/organ). It is hypothesized that persistent gadolinium is not in the form of MP-1177.

Note that 0.3% represents approximately 500 µg/kg of elemental gadolinium. It is predicted that residual gadolinium may be higher in renally impaired subjects.

[¹⁵³Gd]MP-1177/10 was found to distribute to the fetuses of pregnant rats.

Since [¹⁵³Gd]MP-1177/10 was detected in rat milk, it is recommended that nursing mothers receiving Optimark discontinue breast feeding for at least 72 hours following dose administration. (72 hours represents 10 times the elimination half-life of MP-1177/10 in rat milk.)

MP-1177/10 caused reversible blockage of calcium elimination in dogs receiving 28 daily doses of 0.6 or 3.0 mmole/kg. Thirteen to 15 daily doses of 3.0 mmole/kg in a repeat study did not produce the same effect. Single doses of up to 12 mmole/kg did not have an effect on calcium elimination 14 days after dosing and therefore is probably not of clinical concern.

Cardiovascular safety studies in anesthetized dogs demonstrated that MP-1177/10 at doses between 0.3 and 3.0 mmole/kg causes transient, dose-related decreases in heart rate, arterial blood pressure (mean, systolic and diastolic), and left ventricular systolic pressure. Heart rate decreases were slight; blood pressures were decreased by up to %. These effects were not observed at 0.1 mmole/kg which means the NOEL for effects is 0.5 times the human dose of 0.1 mmole/kg based on a body surface area conversion.

The time to peak cardiovascular effects was 30-60 seconds after dosing. Doses below 0.7 mmole/kg returned to baseline by 4 min; however at doses ≥ 0.7 mmole/kg only partial recovery was apparent by the end of the 4 min recording period. However, it can be surmised that cardiovascular effects return to baseline rapidly (within 4 min) at doses up to 0.3 mmole/kg or 1.5 times the human dose.

In these studies, blood pressure dropped without a compensatory increase in heart rate. The Sponsor suggested that the baroreceptor response was blunted by anesthesia and made the point that baroreceptor responses in humans would be intact. The best way to find out if the baroreceptor response was being inhibited in dogs would be to test unanesthetized dogs.

Random arrhythmias and premature ventricular conduction (PVCs) were reported by the Sponsor. They were attributed by the Sponsor to the left ventricular catheter. Because data about the time of occurrence of premature beats were not submitted it is not possible to analyze them to answer questions such as, "Were ventricular conduction later in the study due to predisposition of the heart to early doses in the Latin square design?" It is concluded that the design of this study did not allow a definitive conclusion to be drawn about PVCs.

Effects on PR interval and corrected QT interval were not seen at any dose. The method of QT interval correction was not specified.

A comparison of MP-1177/10 and Magnevist showed that they had similar cardiovascular effects.

Since Optimark is an extracellular agent like approved drugs in its class, and cardiac toxicity is not an issue for this class, and this agent will not be used specifically for cardiac patients, it is recommended that further cardiovascular safety studies in dogs are not requested at this time.

A study of the effects of MP-1177/10 and Magnevist on epinephrine induced contraction of the isolated rat aorta demonstrated no effects at concentrations of 1.5, 5.0 and 15.0 mM. This study suggests that effects observed in dog cardiovascular safety studies (transient decreases in HR and BP) were probably not due to inhibition of sympathetic vascular smooth muscle contraction.

In a battery of 10 pharmacology assays to fulfill Japanese requirements, 3 gave positive results. The findings in mice, decreased spontaneous motor activity lasting 1 hour (NOAEL=0.5 mmole/kg) and prolongation of thiopental anesthesia (NOEL=1.5 mmole/kg), suggested CNS depression. Decreased sodium and chloride elimination and increased urine volume in rats at 5.0 mmole/kg were considered by the Sponsor to be a hyperosmolality effect since the sorbitol control (10 mmole/kg iv) caused the same effects. This is a plausible explanation but not conclusive because these are correlative data from only one source.

The major findings after 4 weeks of daily dosings (up to 3.0 mmole/kg/day) of MP-1177/10T to rats were reversible body weight loss (NOAEL=0.6 mmole/kg); irreversible toxicity to the male reproductive system as evidenced by reduced weight of reproductive organs and histopathological changes (degeneration of the germinal epithelium of the testes, reduced sperm count and germ cells present in epididymides) (NOAEL=0.6 mmole/kg); signs of kidney toxicity such as irreversible increase in kidney weight, urinary pH changes, and reversible dose-related vacuolization of the proximal convoluted tubules (NOAEL=0.6, 0.6, and 0.1 mmole/kg respectively). The data from this multiple dose study suggested that a margin of safety does not exist; therefore, the Sponsor conducted an acute toxicity study with special emphasis on findings from this study.

In the single dose rat toxicity study, only males were tested; the reviewer considers this adequate since effects on the male but not the female reproductive system were found in the 4 week multiple dose study, and males were more sensitive than females to effects other than reproductive effects. Following single doses of MP-1177/10 up to 15 mmole/kg, effects were not observed on body weight and the male reproductive system 24 hours and 14 days after dosing. Effects on the kidneys were found: grossly pigmented kidneys and moderate vacuolization of the proximal tubules 24 hours after

dosing (NOAEL=0.5 mmole/kg); however, signs of recovery were observed 14 days after dosing although recovery was not complete (NOAEL=5.0 mmol/kg or 8 times the clinical dose).

It is concluded that a multiple dosing regimen is needed to produce effects on the rat male reproductive system. For more information, refer to the fertility study discussed later with the reproduction studies.

The major findings after 4 weeks of daily dosings (up to 3.0 mmole/kg/day) of MP-1177/10T to dogs were lethality (max non-lethal dose 0.6 mmole/kg or 3 times the human dose); body weight loss in males (NOAEL=0.1 mmole/kg or no margin of safety); increased alkaline phosphatase (irreversible 8 weeks following the final dose) and decreased phosphorus (reversible) (NOAEL=0); and toxicity to the kidneys as evidenced by reversible cessation of calcium elimination as measured by urinary calcium (NOAEL=0.1 mmole/kg), dose related increases in kidney weights showing partial recovery 8 weeks after the final dose (NOAEL=0.1 mmole/kg), discoloration of kidneys, and reversible vacuolization of the proximal tubular epithelium (NOAEL of kidney effect=0.1 mmole/kg or no margin of safety). Blood chemistry values for the dog sacrificed in moribund condition on the 15th day of daily dosing at 3.0 mmole/kg suggested that renal failure combined with cardiac or liver damage may have been the cause of morbidity.

Note that effects on the male reproductive system found in rats was not observed in dogs.

Single dose toxicity testing in dogs showed that doses up to 12 mmole/kg had no effect on dogs except a slight elevation alkaline phosphatase and a slight decrease in phosphorus levels. Even though these effects were observed at most dose levels, they were still within historical control values. Note that effects on kidney were not observed in this study.

The following table compares maximum non-lethal doses of the 3 currently approved gadolinium agents and Optimark. It appears that Optimark is comparable to the other agents as indicated by this parameter.

Maximum Non-lethal Doses after Single Administration of Gadolinium Agents				
Species	Estimated Multiple of Human Dose of 0.1 mmole/kg*			
	Magnevist	Omniscan	ProHance	Optimark
mouse	7-11	15-17	2.5	8-12
rat	13-42	>8, >33	>16**	>27**
rabbit	53		>20 ***	
dog	>100**		>15**	>60**

*body surface area comparison

**the minimum lethal dose was not reached in the study. The listed value is the highest dose in a study in which deaths were not observed at any dose level.

***lethality to pregnant animals within 24 hrs of the 1st dose in a reproduction study

Regarding the study of the effects of MP-1196 (versetamide) and calcium on the toxicity of MP-1177 (gadoversetamide) in mice, it seems that the objective of such a study should have been to find out the optimal doses of each of these in the final formulation. However, this study did not justify the levels (10%) of MP-1196 and Ca currently added to the MP-1177/10 final formulation. It appeared from the data that the optimal ratio of MP-1196 and calcium to MP-1177 may be <1% , not 10% as was selected for the final formulation.

In a study of the effects of intracisternal administration of Optimark on toxicity to the rat brain, the reviewer found that it was difficult to compare rat brain exposure following an intracisternal dose to possible brain exposure in humans following an iv dose without making a lot of assumptions. However, it was found that the margin of safety for clinical signs of neurotoxicity (such as dyspnea, hypoactivity, convulsions, tremors, rearing pawing) is at least 21. This safety factor was estimated using conservative assumptions in terms of safety about the brain kinetics of MP-1177 in rats and humans.

In another intracisternal study in rats for neurotoxicity effects, MP-1177/10 administration did not affect body weights or a different set of measures than the previous study: rotarod performance and histopathological effects on brain, spinal cord, spinal nerve roots, or dorsal root ganglia. The high dose in this study, 100 umole/kg, was estimated to produce a CSF concentration at least 40 times the estimated brain concentration in patients with disrupted blood:brain barrier.

The major findings from reproductive toxicology studies conducted in rats and rabbits were:

A. Fertility And Reproductive Performance Study

1) toxicity to the male reproductive system of rats at daily doses of 2.0 mmole/kg for 7 weeks as evidenced by irreversible reduction and degeneration of spermatocytes in testes and epididymides, and reduced male fertility in these animals. These effects were not found at lower doses (NOAEL=0.5 mmole/kg or 1 times the human dose based on a body surface area comparison).

2) reduced body weight at birth through weaning in rats at daily doses of 0.5 mmole/kg. This effect was not found at the lower dose (NOAEL=0.1 mmol/kg or 0.2 times the human dose based on a body surface area comparison.)

B. Developmental Toxicity Studies

2) retarded growth, abnormal liver lobation and delayed ossification of sternbrae of F₁ fetal rats from F₀ females dosed with 4.9 mmole/kg (NOAEL=0.7 mmole/kg or 1 times the human dose)

3) forelimb flexures and cardiovascular changes in F₁ rabbits from F₀ females dosed with 0.4 and 1.6 mmole/kg. The cardiovascular changes were malformed thoracic arteries, septal defect, and abnormal ventricle. (NOAEL=0.1 mmole/kg or 0.3 times the human dose)

Note: Although incidences of forelimb flexures were within historical controls for the conducting laboratory, the controls from this study had a lower incidence than treated groups. However, a preliminary study gave similar results. Therefore, cardiovascular changes and forelimb flexures are both considered treatment-related effects.

Findings from reproductive toxicology studies should be reported in the labeling.

The Sponsor has conducted an adequate array of genotoxicity studies by FDA standards (see ICH S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals). According to this standard, the battery should consist of at least 3 tests. These recommended tests are listed below with the studies the Sponsor conducted to comply with the recommendations:

ICH S2B Recommended Test	Test Conducted by Sponsor which fulfills ICH S2B Recommendation
A test for gene mutation in bacteria	Salmonella / Mammalian Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and Escherichia Coli WP2 UVRA Reverse Mutation Assay with a Confirmatory Assay
An in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells or an in vitro mouse lymphoma tk assay	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells and L5178Y TK +/- Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay
An in vivo test for chromosomal damage using rodent hematopoietic cells	Micronucleus Cytogenetic Assay in Mice

All tests were negative except the Chromosome Aberrations in CHO cells assay. This assay was positive at the highest concentration 5000 ug/ml (7.6 umole/ml) in cultures not activated with S-9. This positive result should be reported in the labeling.

Local irritation studies included venous irritation, intramuscular and subcutaneous irritation studies, all conducted in rabbits. The venous irritation studies were conducted under exaggerated conditions: 0.5 M MP-1177/10 was injected into a clamped section of the vein and kept there for 3 min; this was done 16 times over 3 days. Under these conditions treated animals showed a mild reaction (mild phlebitis and periphlebitis). However, mild necrosis and perivascular necrosis was also observed in 1 of 3 animals in one study and thrombosis and necrosis comparable to the positive control was found in 1 of 3 animals from the other study. The Sponsor concluded that MP-1177/10 was mildly irritating to veins. In the reviewers opinion, it is more than mildly irritating under the conditions of this study. It would be useful to know if the more significant effects could have been avoided by reducing the number of doses.

Results of intramuscular and subcutaneous irritation studies were negative when the equivalent of 0.2 mmole/kg was injected into im and sc sites.

Data from human blood compatibility studies support that MP-1177/10 is compatible with human blood.

In a screen of antigenicity tests, it was concluded that MP-1177/10 was very weakly antigenic. This was based on results of a guinea pig active systemic anaphylaxis (ASA) assay in which "sparse reactions such as rubbing or licking the nose and ruffling the fur" were observed. Negative assays included homologous passive cutaneous anaphylaxis (PCA) and passive hemagglutination (PHA) assays in guinea pigs, and heterologous PCA assays (serum from 2 strains of sensitized mice tested in rats).

MP-1177/10 produced concentration-related relaxation of histamine- and acetylcholine-induced contractions of the isolated guinea pig trachea. Since the responses were similar in the histamine and acetylcholine systems, it is concluded that MP-1177/10 does not inhibit smooth muscle contraction by antagonism of these receptors. The effect of MP-1177/10 was similar to Magnevist in this assay. This effect would not be expected at the proposed human dose level of 0.1 mmole/kg.

Labeling Review

Recommended changes to the package insert follow:

1) Page 2, under "DISTRIBUTION"

The second sentence currently reads:

Please change it to the following:

In pregnant and lactating rats which received ¹⁵³Gd-labeled gadoversetamide, radioactivity was detected in the placenta, fetus and milk. (See the PREGNANCY CATEGORY C and NURSING MOTHERS sections).

2) Page 6, the 2nd paragraph under "PRECAUTIONS General"

Delete the following sentences:

And replace with:

Animal data demonstrated that insignificant levels of radioactive [¹⁵³Gd] MP-1177/10 are eliminated via the feces. In experimentally induced anephria in the rat, hepatobiliary excretion did not significantly compensate for blockage of

urinary elimination. Therefore, dialysis may be needed to clear Optimark if it is administered to a renally impaired subject.

3) Page 7, under CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Delete the genotoxicity statement:

And replace it with:

The results of the following genotoxicity assays were negative: Salmonella/E. coli reverse mutation (Ames) assay, mouse lymphoma mutagenesis assay, and the in vivo mammalian micronucleus assay. The CHO chromosome aberration assay was positive only at the highest concentration, 5000 ug/ml (7.6 umole/ml), without metabolic activation.

Delete the impairment of fertility statement:

And replace it with:

Optimark caused irreversible reduction and degeneration of spermatocytes in the testes and epididymides of rats, and impaired male fertility following daily intravenous doses of 2.0 mmole/kg for 7 weeks. These effects were not observed at 0.5 mmole/kg or 1 times the human dose based on a body surface area comparison.

In a separate 28-day repeat dose study, Optimark caused irreversible reduction of male reproductive organ weights, degeneration of the germinal epithelium of the testes, presence of germ cells in the epididymides, and reduced sperm count, following daily intravenous doses of 3.0 mmole/kg. These effects were not observed at 0.6 mmole/kg or 1 times the human dose.

In a single dose study, Optimark did not produce adverse effects on the male reproductive system 24 hours and 14 days after intravenous administration of up to 15 mmole/kg or 25 times the human dose.

4) Page 8, under PREGNANCY CATEGORY C

Delete the pregnancy statement as written:

And replace with:

Optimark caused a reduction in neonatal weights from birth through weaning at maternal doses of 0.5 mmole/kg for 5 weeks (including gestation) and paternal doses of 0.5 mmole/kg for 12 weeks. This effect was not observed at 0.1 mmole/kg or 0.2 times the human dose based on a body surface area comparison. Maternal toxicity was not observed at 0.5 mmole/kg.

Optimark caused retarded growth, abnormal liver lobation, delayed ossification of sternebrae, and delayed behavioral development in fetuses from female rats dosed with 4.9 mmole/kg on days 7-17 of gestation. These effects were not observed at 0.7 mmole/kg or 1 times the human dose. Maternal toxicity was observed at 4.9 mmole/kg.

Optimark caused forelimb flexures and cardiovascular changes in fetuses from female rabbits dosed with 0.4 and 1.6 mmole/kg on gestation days 6 through 18. The cardiovascular changes were malformed thoracic arteries, a septal defect, and abnormal ventricle. These effects were not observed at 0.1 mmole/kg or 0.3 times the human dose. Maternal toxicity was not observed at any dose.

5) Page 8, under NURSING MOTHERS

Delete the nursing mothers statement as written:

cc:

Orig. NDA 20-937

HFD-160/Division File

HFD-160/Pharm/Melograna

HFD-160/Chem/Place

HFD-160/MO/Raman

HFD-160/MO/Yaes

HFD-160/CSO/Moore

HFD-870/Biopharm/Choi

HFD-345