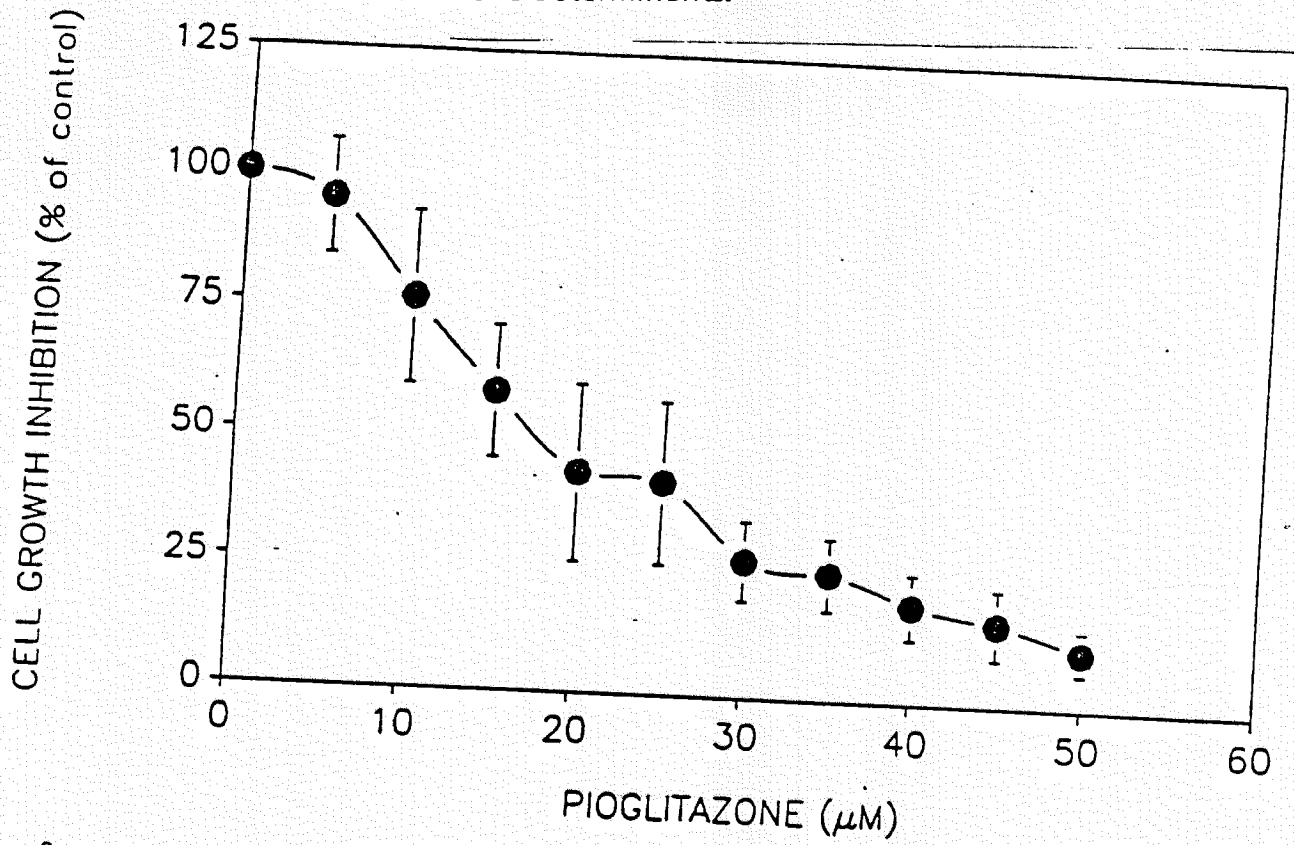
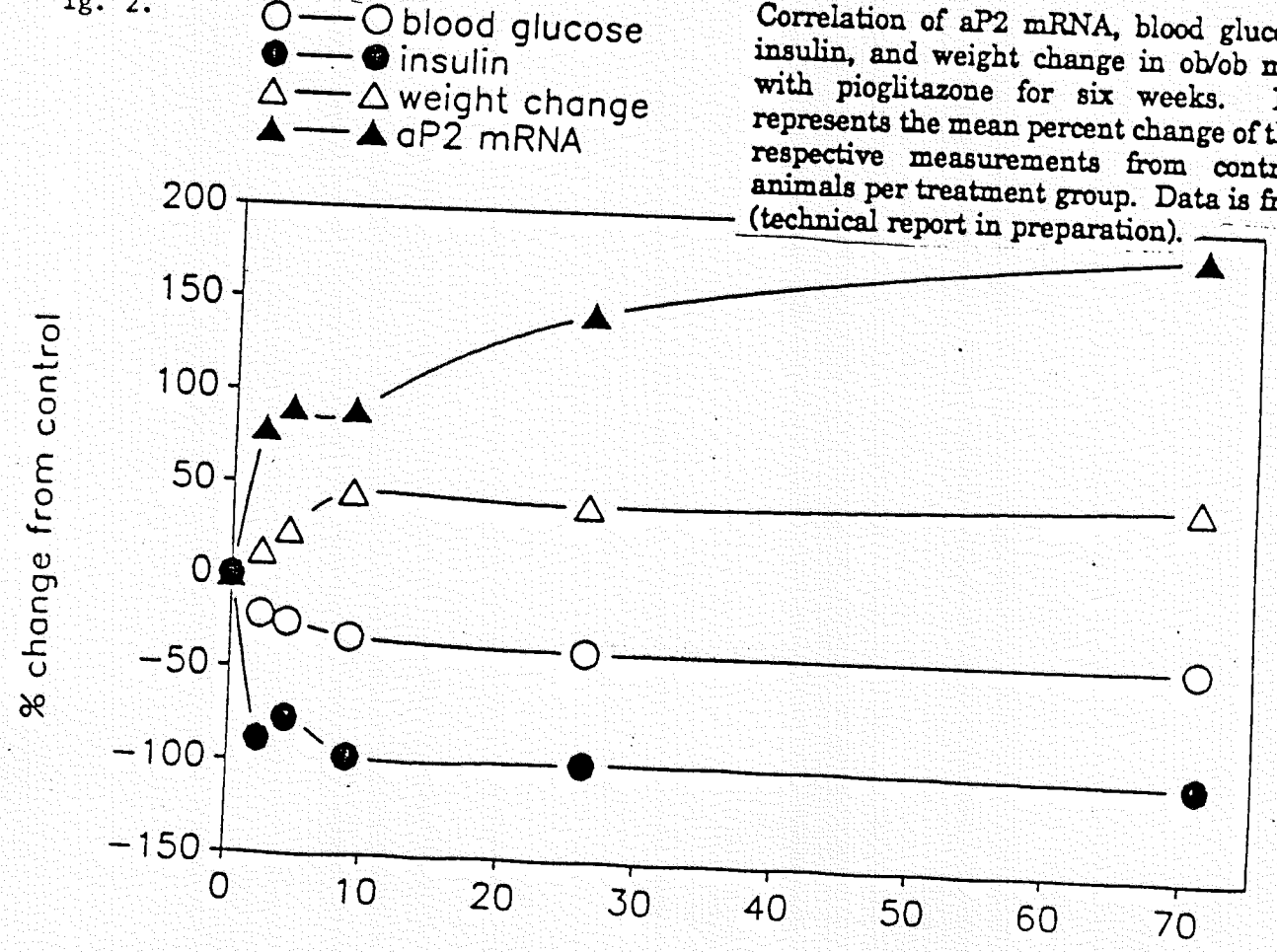
FIGURE 1. Dose Response: H4-II-E cells plated 6-7 hours were treated for 96 hours with pioglitazone. Cells were analyzed by protein or number, and the data plotted as percent of control (control = 100%). The IC_{50} was 19.5 µM. Each point represents the mean ± S.E. of 8 determinants.



ig. 2. Correlation of aP2 mRNA, blood glucose,. plasma insulin, and weight change in ob/ob mice treated with pioglitazone for six weeks. Each point represents the mean percent change of the values of respective measurements from control for six animals per treatment group. Data is from B.Wyse (technical report in preparation).



H. Rhee

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

Mr. Markus F. Herzig
Takeda America, Inc.
101 Carnegie Center
Princeton, NJ 08540

July 3, 1996

Dear Mr. Herzig:

Re: IND [redacted]/Pioglitazone HCl
Amendment [redacted]

On July 19, 1995 our division had a meeting with you for the transfer of IND# [redacted] from Upjohn to Takeda America, Inc. During the meeting two ongoing carcinogenicity studies were discussed and Dr. Jordan requested the justification of the dose levels used for the studies.

In response to our request, you indicated that the two carcinogenicity studies [redacted] #295-153 and [redacted] #295-152) were completed [redacted]. Based on your preliminary analysis of the studies, the incidence of focal proliferative lesions in the urinary bladder in rats was reported subsequently on June 20, 1996 [redacted].

We have completed our review and have the following requests:

1. Please submit the final results from the two carcinogenicity studies as soon as possible.

2. Since we have not examined your cancer study protocols, please provide justification of dose selection including AUC comparisons in test animals and human exposure.

3. In addition, comparative metabolic profile of pioglitazone in the three species will help in determing the relevance of animal findings to human exposure.

Sincerely,

/S/ [redacted]
Herman Rhee, Ph.D.

/S/ [redacted]
Ronald W. Steigerwalt, Ph.D.

/S/ [redacted] 7/2/96
Solomon Sobel, M.D.
Division Director

cc: Orig. IND
HFD-510/Div File
HFD-510/HRhee/RSteigerwalt
HFD-510/HRhee

APPEARS THIS WAY ON ORIGINAL

H. Rhee

DEC 3 1996

IND# [redacted]

December 2, 1996

Sponsor: Takeda America, Inc., Princeton, NJ. Markus F. Herzig
Tel(609)452-1113; Fax(609)452-1218

Amendment Serial No. 071
Submission Date: Nov. 4, 1996

Drug: Pioglitazone HCl(U-72,107A, AD-4833)

Class: Oral hypoglycemic agent

Related: IND [redacted]
IND
IND
IND

This amendment contains the sponsor's response to our request of July 3, 1996 on their carcinogenicity studies([redacted] #295-153 and [redacted] #295-152). The sponsor performed metabolic studies in rats, mice, and human, using radioactive pioglitazone.

A. Methods: Unspecified number of male Jcl:Sprague Dawley rats and male Crj:ICR mice were administerd 0.5 mg/kg of [redacted]C-Pioglitazone. Pioglitazone-HCl tablet(30 mg) was given to human male volunteers. Blood, urine and feces were collected for the determination of pioglitazone and its metabolites by [redacted]

B. Results: Table 1 shows the composition of metabolites in rat and mouse plasma, and human serum. Cumulative excretion of pioglitazone in urine and feces is summarized in table 2. Postulated metabolic pathways of pioglitazone is shown(Fig. 1).

C. Attachment: Two tables and one figure.

D. Recommendations: (Letter to the sponsor)

1. Are the 6 metabolites active? If they are, please indicate the relative activity ratios in three species, mouse, rat and human. Are the two stereoisomers equally active?

2. Please repeat the experiments in Crl:CD BR rats and Crl:CD-1 mice with appropriate sample size since they were used in your two-year carcinogenic studies.

/S/ [redacted]

Herman M. Rhee, Ph. D.

/S/ [redacted] 12/3/96

cc: Original IND, HFD-510
Ronald Steigerwalt/H. Rhee

[redacted]
APPEARS THIS WAY ON ORIGINAL

Table 1
Composition of metabolites in rat and mouse plasma, and human serum after oral administration of pioglitazone (HCl)

Compound	Rat		Mouse		Human	
	AUC(0-48h)	R(A)	AUC(0-48h)	R(A)	AUC(0-96h)	R(A)
Pioglitazone	7.22±1.11	1.00	3.00	1.00	13.11±3.59	1.00
M-I	0.04±0.02	0.01	0.01	<0.01	0.30±0.92	0.04
M-II	1.30±0.74	0.18	0.31	0.10	0.68±0.27	0.05
M-III	1.97±0.20	0.27	0.25	0.08	11.65±2.98	0.86
M-IV	1.28±0.08	0.18	0.49	0.16	24.81±4.38	1.81
M-V	0.60±0.07	0.08	0.15	0.05	0.92±0.20	0.06
M-VI	0.12±0.05	0.02	0.05	0.02	1.16±0.22	0.08
R(B)	92.6%		86.4%		****	

AUC: Mean ± SD (n= 5 for rats and n= 14 for humans)
AUC for mice was calculated using the mean plasma concentration (n=3) at each time.
Dosing: [■C]pioglitazone (HCl) 0.5 mg/kg for rats and mice,
pioglitazone (HCl) 30 mg/man (as pioglitazone) for humans
AUC: pioglitazone eq μg.h/ml for rats and mice, and μg.h/ml for humans
R(A):AUC of each metabolite /AUC of pioglitazone
R(A) for humans was calculated using AUC each corrected by the molecular weight of each metabolite to that of pioglitazone
R(B):(total AUC of pioglitazone and M-I to M-VI/AUC of total radioactivity) x 100
****:not calculated

Table 2

Cumulative excretion of pioglitazone and its metabolites in rats, mice and humans after oral administration of pioglitazone (HCl)

% of dose excreted

Compound	Rat						Mouse						Human		
	Urine (0-48h)			Feces (0-48h)			Urine (0-48h)			Feces (0-48h)			Urine (0-48h)		
	UC	CON	Total	UC	CON	Total	UC	CON	Total	UC	CON	Total	UC	CON	Total
Pioglitazone	N.D.	N.D.	N.D.	1.7	N.D.	1.7	N.D.	0.1	0.1	1.0	0.2	1.2	N.D.	N.D.	N.D.
M-I	0.1	1.7	1.8	N.D.	0.2	0.2	0.1	0.9	1.0	0.2	0.1	0.3	N.D.	0.5	0.5
M-II	N.D.	N.D.	N.D.	2.7	N.D.	2.7	0.1	3.7	3.9	0.3	N.D.	0.4	N.D.	0.7	0.7
M-III	N.D.	N.D.	N.D.	0.2	N.D.	0.2	N.D.	0.1	0.1	0.1	0.1	0.1	N.D.	N.D.	N.D.
M-IV	0.1	1.9	2.0	1.8	8.9	10.7	N.D.	0.6	0.6	0.6	1.8	2.3	N.D.	7.8	7.8
M-V	9.5	1.4	10.9	13.7	2.4	16.1	1.2	0.8	2.0	2.5	1.6	4.1	11.8	0.8	12.6
M-VI	2.8	0.1	2.9	4.0	0.3	4.3	0.3	0.2	0.5	7.9	N.D.	7.9	7.8	N.D.	7.8
Others			17.9			25.8			15.7			58.6			****
Total	35.5±0.5			61.7±1.9			23.9±5.9			74.9±7.7			29.3±5.9		

Mean ± SD or Mean (n=3 for rats, n=3 for mice and n=13 for humans)

Dosing: [[redacted]C]pioglitazone (HCl) 0.5 mg/kg for rats and mice
pioglitazone (HCl) 30 mg/man (as pioglitazone) for humans

****: not assayed

N.D.: not detected

UC: unconjugate

CON: conjugated form (glucuronide or sulfate, except for M-V in rats and mice which contains taurine conjugate)

Total: total radioactivity for rats and mice (including "others") and total of pioglitazone and metabolites (M-I to M-VI) for human urine

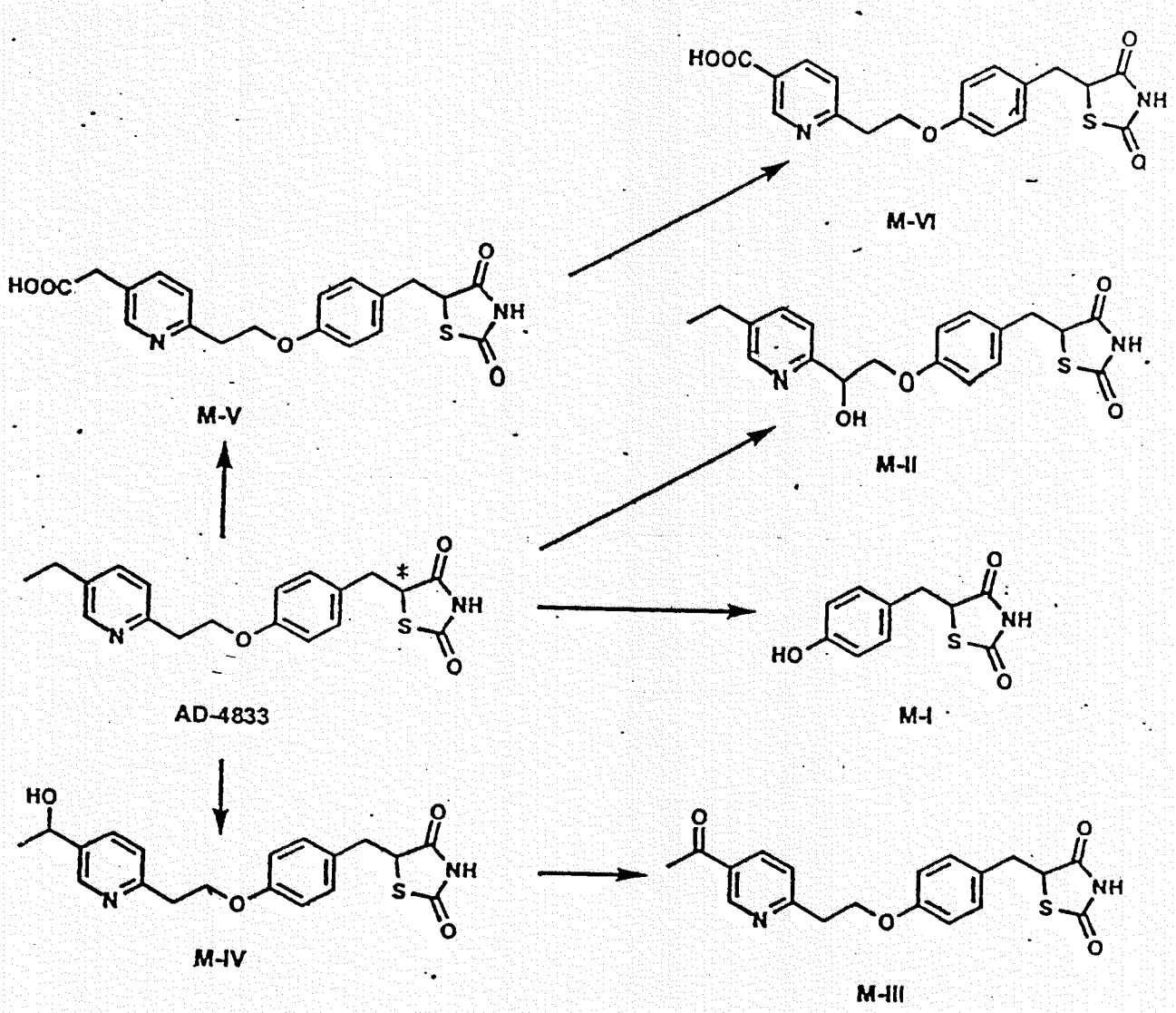


Fig. 1 Postulated metabolic pathways of pioglitazone

*: [redacted]C-labeled position

IND# [redacted]

June 23, 1997

Sponsor: Takeda America, Inc., Princeton, NJ. Markus F. Herzig
Tel(609)452-1113; Fax(609)452-1218

Amendment Serial No. 101
Submission Date: 6/5/1997

Drug: Pioglitazone HCl(U-72,107A, AD-4833)

Class: Oral hypoglycemic agent

Related: IND# [redacted]
IND#
IND#
IND#

In this amendment the sponsor wishes to respond to the points that were raised by the reviewer on September 18, 1996. The main points are summarized below.

1). In order to characterize the metabolites of pioglitazone the sponsor quantitated them in plasma of several species after oral administration, of which results are summarized below.

Met[@]	Mouse		Rat		Dog		Monkey		Human	
	AUC	RA	AUC	RA	AUC	RA	AUC	RA	AUC	RA
Pio'	3.00	1.00	7.22	1.00	0.89	1.00	5.55	1.00	13.1	1.00
M-1	0.01	0.01	0.04	0.04	0.12	0.13	0.10	0.02	0.30	0.02
M-2	0.31	0.10	1.30	0.18	0.19	0.21	0.36	0.06	0.68	0.05
M-3	0.25	0.08	1.97	0.27	0.43	0.48	0.40	0.07	11.6	0.29
M-4	0.49	0.16	1.28	0.18	2.41	2.71	2.87	0.52	24.8	1.89
M-5	0.15	0.05	0.60	0.08	0.30	0.34	0.93	0.17	0.92	0.07
M-6	0.05	0.02	0.12	0.02	ND	ND	ND	ND	1.16	0.09
RB	86.4%		92.6%		79.0%		93.1%		NC	

[@] Met or M stand for metabolites and ' indicate parent compound, respectively. AUC represents values from 0 to 24 hrs. RA represents AUC ratio of each metabolite to pioglitazone at dose of 0.5 mg/kg. For Human dose was 30 mg. RB shows AUC ratio(total metabolites to total radioactivity)x100. ND and NC stand for not determined and not calculated, respectively.

2). As to the minimum dose of pioglitazone which causes cardiac enlargement, the sponsor provided the followings.

Species	Duration of dose	Sex	Dose level (mg/kg/day)	Mean AUC_{0-24h} (μg.hr/ml)	Dose ratio vs human	Exposur ratio vs human
Rat	1-year	Male	3.6	70.4	7	4.6
		Female	14.5	234	29	15.3
Mouse	2-year	Male	27.0	110	54	7.2
		Female	90.0	287	180	18.8
Dog	1-year	Male	3	9.4	6	0.6
		Female	10	14.5	20	1.0
Monkey	3-month	Male	8	75.3	16	4.9
		Female	8	74.4	16	4.8

*Human dose was 30mg/day(0.5mg/kg/day), which gave an AUC value, 15.3μg.hr/ml.

3). The sponsor noted benign and/or malignant transitional cell tumors in the urinary bladder at dose of 3.6 mg/kg/day in 2-year rat carcinogenicity study. Mean plasma $AUC_{(0-24\ hr)}$ of pioglitazone was 61.2μg.hr/ml. The AUC ratio of rat/human was only 4.

4). The sponsor attached a figure to explain the mode of pioglitazone hypoglycemic action, although the exact mechanism of its action remains to be defined.

5). Finally the sponsor attached an unpublished paper of which title is "Pharmacological activities of pioglitazone.HCl and its metabolites". They compared hypoglycemic action of pioglitazone and its metabolites in Wistar fatty rat. The results are:

Metabolites	Pio[e]	M-1	M-2	M-3	M-4	M-5	M-6
Plasma glucose							
ED_{25}(mg/kg/d)	0.54	3.00	0.99	1.32	0.93	3.00	3.00
Relative Pot[r]	1.00	0.18	0.55	0.41	0.58	0.18	0.18
Plasma triglyceride							
ED_{25}(mg/kg/d)	0.43	3.00	0.22	0.47	0.53	3.00	3.00
Relative Pot[r]	1.00	0.18	1.95	0.91	0.81	0.18	0.18

Pio[e] and relative pot[r] indicate pioglitazone and relative potency,

respectively. Potency of pioglitazone was set as 1.00.

6). Summary and Conclusion:

The dog has the closest metabolite profile to human. The rank order for hypoglycemic action was Pioglitazone>M-4>M-2>M-3>M-1=M-5=M-6, while that of its effect on triglyceride was M-2>Pio>M-3>M-4>M-1=M-5=M-6 in Wistar rats. The rank of AUC ratio at the minimum dose that caused cardiac enlargement was mouse>rat>monkey>dog. The AUC ratio in female dog was identical to human, while that in male dog was 0.6. The AUC ratio of rat/human at a dose of 3.6mg/kg/day was 4 when benign and/or malignant transitional cell tumors in the urinary bladder were noted.

7). Attachment: A figure.

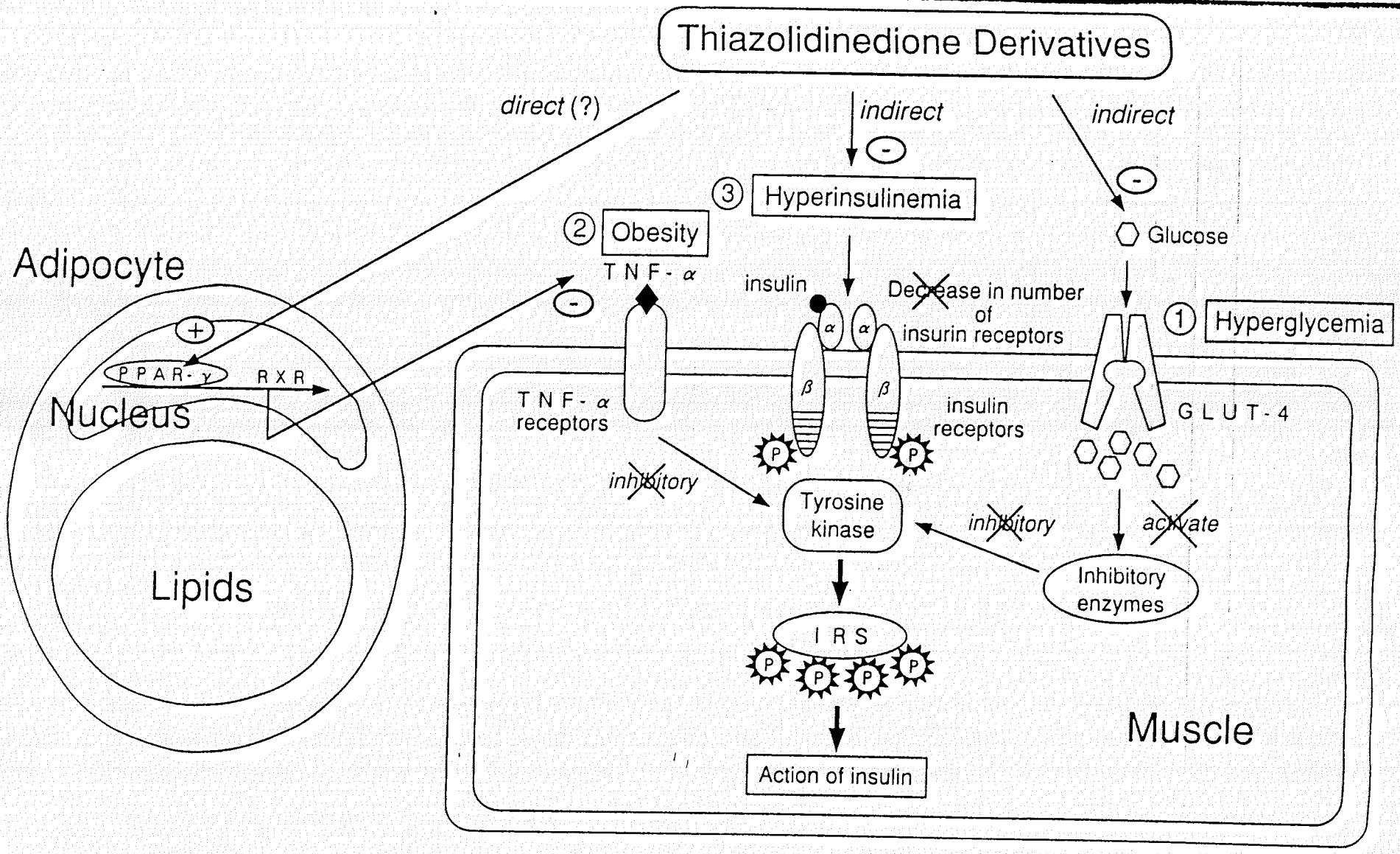
8). Recommendation: None.

/S/ [redacted]

Herman M. Rhee, Ph. D.

cc: Original IND, HFD-510
Ronald Steigerwalt/H. Rhee

[redacted]
APPEARS THIS WAY ON ORIGINAL



Possible Mechanisms of Thiazolidinedione Derivatives

IND# [redacted] July 28, 1997

Sponsor: Takeda America, Inc., Princeton, NJ. Markus F. Herzig
Tel(609)452-1113; Fax(609)452-1218

Amendment Serial No. 101
Submission Date: 6/5/1997

Drug: Pioglitazone HCl(U-72,107A, AD-4833)

Class: Oral hypoglycemic agent

Related: IND# [redacted]
IND#
IND#
IND#

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Pio'	3.00	1.00	7.22	1.00	0.89	1.00	5.55	1.00	13.1	1.00
M-1	0.01	0.01	0.04	0.04	0.12	0.13	0.10	0.02	0.30	0.02
M-2	0.31	0.10	1.30	0.18	0.19	0.21	0.36	0.06	0.68	0.05
M-3	0.25	0.08	1.97	0.27	0.43	0.48	0.40	0.07	11.6	0.29
M-4	0.49	0.16	1.28	0.18	2.41	2.71	2.87	0.52	24.8	1.89
M-5	0.15	0.05	0.60	0.08	0.30	0.34	0.93	0.17	0.92	0.07
M-6	0.05	0.02	0.12	0.02	ND	ND	ND	ND	1.16	0.09
RB	86.4%		92.6%		79.0%		93.1%		NC	

@ Met or M stand for metabolites and ' indicate parent compound, respectively. AUC represents values from 0 to 24 hrs. RA represents AUC ratio of each metabolite to pioglitazone at dose of 0.5 mg/kg. Human dose was 30 mg. RB shows AUC ratio(total metabolites to

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*Human dose was 30mg/day(0.5mg/kg/day), which gave an AUC value, 15.3µg.hr/ml. #Calculated based on AUC of parent compound only.

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ED_{25}(mg/kg/d)	0.43	3.00	0.22	0.47	0.53	3.00	3.00
Relative Pot[f]	1.00	0.18	1.95	0.91	0.81	0.18	0.18

Pio[e] and relative pot[f] indicate pioglitazone and relative potency, respectively. Potency of pioglitazone was set as 1.00.

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7). Attachment: A figure.

8). Recommendation: (Letter to the sponsor)

The amendment(Serial No. 101) deals with important pharmacokinetic issues. Animals have different levels of metabolites I through VI and the metabolites have different pharmacological activities. Therefore, it is necessary to calculate animal exposure ratio vs human, based on AUC ratio of parent drug and all relevant active metabolites. Please compare the exposure ratio in this way at the minimum dose of pioglitazone which causes cardiac enlargement and bladder tumors.

/S/ [redacted]

Herman M. Rhee, Ph. D.

cc: Original IND, HFD-510
Ronald Steigerwalt/H. Rhee/M. Johnston