CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 22-156

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22156
SUBMISSIONS REVIEWED	Amendements dated
	4/25/2008
	5/30/2008
PRODUCT:	Cleviprex™ (clevidipine butyrate injectable emulsion)
INTENDED CLINICAL POPULATION:	[redacted]
SPONSOR:	**The Medicines Company**
REVIEW DIVISION:	**Division of CardioRenal Drug Products (HFD110)**
PHARM/TOX REVIEWER:	**E.A. Hausner, D.V.M.**
PHARM/TOX SUPERVISOR:	**Charles Resnick, Ph.D.**
DIVISION DIRECTOR:	**N. Stockbridge, M.D., Ph.D.**
PROJECT MANAGER:	**Alisea Crowley**

PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION AND DRUG HISTORY

NDA number: 22156

Submission type and date Amendments dated April 25, 2008 (receptor binding studies)
May 30, 2008 (genotoxicity studies)
Sponsor and/or agent: The Medicines Company

Reviewer name: Elizabeth Hausner, D.V.M.
Division name: **Division of Cardio-Renal Drug Products**
HFD #:110
Review completion date: June 19, 2008

Drug:

Trade name: Cleviprex™
Generic name: clevidipine butyrate
Code name: H324/38

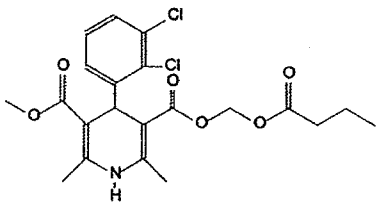
Chemical names:

Butyroxymethyl methyl 4-(2',3'-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate

4-(2',3'-dichlorophenyl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 3-butyryloxymethyl ester 5-methyl ester

CAS registry number: 167221-71-8
Molecular formula/molecular weight:

$C_{21}H_{23}Cl_2NO_6$



2930.D

Structure:

Related application: Medicines Company IND 65,114

Drug class: dihydropyridine calcium channel blocker

Studies Submitted:

Receptor screening study for clevidipine and its primary metabolite H152/81(M1)

Clevidipine with and without degradants ([redacted] Bacterial Mutation Test (Ames Test, Report Number 962193)

Clevidipine with and without degradants ([redacted] Chromosome Aberration Test (Report Number 962194)

Studies Reviewed:

Receptor screening study for clevidipine and its primary metabolite H152/81(M1)

Clevidipine with and without degradants ([redacted] Bacterial Mutation Test (Ames Test, Report Number 962193)

Clevidipine with and without degradants ([redacted] Chromosome Aberration Test (Report Number 962194)

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Background

Two submissions are reviewed herein. The material was sent to the Division in response to:

February 25, 2008 Pharmacology/Toxicology and CMC Discipline Review Letter

The CMC Discipline review letter requested a scientific justification for the sponsor's proposed limits of ———) each for ——— , which each had structural alerts for genotoxicity. The limits proposed were — imes (per genotoxin) the EMEA's Threshold of Toxicological Concern (TTC).

March 6, 2008 Pharmacology/Toxicology and CMC Discipline Review Letter

The Division requested the sponsor to address the potential genotoxicity of formaldehyde in combination with drug product ———

The Division requested a receptor binding profile of clevidipine butyrate.

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ON ORIGINAL

Receptor screening study for clevidipine and its primary metabolite H152/81(M1)

Study location: [redacted]
Report Number: 08-4680
Study initiated: March 6, 2008
GLP: no statement included
QA: not found
Test article: clevidipine butyrate, lot # 2930.D.03.601, purity 99.81% by HPLC
H152/81 lot # 2930.C.03.602, purity 98.1% by HPLC

The material submitted is a receptor binding screen in response to my request for this information.

The concentrations of clevidipine butyrate and H152/81 (main metabolite) tested were 1 nM, 100 nM and 10 μM. The sponsor chose these concentrations for the clinical relevance:

> **The concentration range selected for the study brackets the expected therapeutic (clinically relevant) concentrations of clevidipine (about 50 nM at 16 mg/h and about 100 nM at the maximum dose of 32 mg/h) and H152/81 metabolite concentrations (concentration at steady state is estimated to be about 30 μM when clevidipine is infused at the maximum dose of 32 mg/h) when clevidipine is administered at the maximum proposed dose and duration. Many patients are effectively treated at lower doses and for shorter duration of time.**

The initial presentation of data lists results for the test compound as Kd while the reference compound results are presented as Ki. Tables listing percent inhibition of binding were also found.

Clevidipine shows some binding to the adenosine A_{2A} receptor (human recombinant)

Client Name: The Medicines Company		Barcode Number:	084680-1
Client Contact: [redacted]		Client Number:	Clevidipine
Task Order Number: 08-4680		Solubility of Stock:	Soluble
	Percent Inhibition (Average; N= 3)		
Receptor	1.0E-9 M	1.0E-7 M	1.0E-5 M
NEUROTRANSMITTER RELATED			
Adenosine Transporter (h)	-2.21%	-1.08%	7.51%
Adenosine, A1	-15.33%	-7.20%	28.46%
Adenosine, A2A (h)	**-16.36%**	**-9.18%**	**64.82%**

As expected there was concentration related binding of clevidipine to calcium channels.

ION CHANNELS			
Calcium Channel, Type L (Dihydropyridine Site)	35.44%	73.98%	106.87%

Clevidipine also showed a degree of binding to the thromboxane A2 (TXA2) receptor.

Client Contact: [redacted] Client Number: Clevidipine
Task Order Number: 08-4680 Solubility of Stock: Soluble

Receptor	Percent Inhibition (Average; N= 3) 1.0E-9 M	1.0E-7 M	1.0E-5 M
PROSTAGLANDINS			
Leukotriene, LTD4 (CysLT1)	-9.20%	-17.33%	-9.18%
Thromboxane A2 (h)	-11.56%	9.26%	84.19%

The metabolite H152/81 also showed concentration related binding to the L-type calcium channel as well as binding to the TXA2 receptor.

Client Contact: [redacted] Client Number: H152/81
Task Order Number: 08-4680 Solubility of Stock: Soluble

Receptor	Percent Inhibition (Average; N= 3) 1.0E-9 M	1.0E-7 M	1.0E-5 M
ION CHANNELS			
Calcium Channel, Type L (Dihydropyridine Site)	17.82%	18.95%	86.55%

Client Contact: [redacted] Client Number: H152/81
Task Order Number: 08-4680 Solubility of Stock: Soluble

Receptor	Percent Inhibition (Average; N= 3) 1.0E-9 M	1.0E-7 M	1.0E-5 M
PROSTAGLANDINS			
Leukotriene, LTD4 (CysLT1)	3.29%	7.47%	6.62%
Thromboxane A2 (h)	7.64%	0.99%	84.75%

The remainder of the material submitted included summaries of hemorrhagic adverse events in clinical trials and cardiac adverse events. This has been brought to the attention of the medical reviewer. I agree with the sponsor that at this stage, the clinical data is the best place to investigate the possible implications of the findings of clevidipine binding to the TXA2 receptor.

The sponsor also examined non-clinical data for prolonged bleeding, thromboembolic events and bronchoconstrictive events as these are the major effects associated with modulation of the TXA2 receptor. The toxicological data reviewed included studies in rats and dogs extending to 28 days duration. In the repeat dose studies clevidipine was administered as a continuous IV infusion for 12 or 24 hours per day at a dose range of 6.8 to 66 mg/kg/day in most studies. The maximum anticipated human dose is 32 mg/hour. In the rat 4 week study at the HD of 66 mg/kg/day, steady state blood levels of clevidipine were approximately 86 nM and for H152/81 were 216μM. In the dog 4 week study, the HD of 66 mg/kg/day produced steady state blood concentrations of 972nM clevidipine and 6.75μM H152/81.

Tabular summary of blood levels of clevidipine and H152/81

Species/study	Dose	Blood concentrations	
		clevidipine	H152/81
Rat 4 week study	66 mg/kg/day	86nM	216μM
Dog 4 week study	66 mg/kg/day	972nM	6.75μM
Human	32 mg/hour	50-100 nM	10-30μM

There were no reports of untoward bleeding in the rat and dog toxicology studies. There were no consistent changes in prothrombin time (PT) or activated partial thromboplastin time (aPTT) in the studies examined.

The conclusion from the non-clinical view is that clevidipine also shows binding to the adensosine A_{2A} and thromboxane A2 receptors. As is usual in these types of screening studies, we do not know if the result of the receptor ligand interaction is agonism or antagonism, competitive or non-competitive, reversible or irreversible. The most appropriate data for sorting the potential significance of the findings is in the clinical material, as already provided.

Clevidipine with and without degradants [redacted] ***: Bacterial Mutation Test***

Study location. [redacted]

Report Number: 962193
GLP: statement included
QA: yes
Study dates: experimental phase April 15-April 18, 2008
Test articles: see below
Bacterial strains used: *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, TA102
Escherichia coli strain WP2*uvrA*

Positive controls (-S9): sodium azide, 9-aminoacridine (9AC), 2-nitrofluorene(2NF),4-Nitroquinoline N-oxide (NQO), mitomycin C(MMC)

Positive controls (+S9): 2-aminoanthracene (2AA), benzo[a]pyrene (BaP)
S9 mix: purchased from Moltox™. Made from liver of male SD rats treated with Phenobarbital/5,6-benzoflavone.

Formaldehyde dehydrogenase (FDH): from Pseudomonas putida and dissolved in sodium phosphate buffer to a final activity of 4 units/ml for use with TA98 and 40 units/ml for use with TA100 and TA102. The concentrations were reported as optimized for detoxification of formaldehyde based on previous work at the testing facility.

Vehicle: DMSO

Sterility and vehicle controls: The sterility of the buffer, S9 mixes, FDH solutions, vehicle for test article formulation, reference article (high dose), positive controls (high dose where more than one dose was tested) and the test article formulation (high dose) were assessed on the day of the test using appropriate preparations without bacteria.

The spontaneous mutation rates of the bacterial strains were assessed using concurrent control samples in which the bacteria were exposed to the vehicle.

The bacterial strains listed were treated with the test articles at a range of concentrations up to 5000 μg/plate ±S9 mix using the plate incorporation and pre-incubation versions of the bacterial mutation test. In addition, strains TA98, TA100 and TA102 were treated in the presence of S9 mix with formaldehyde dehydrogenase (FDH) using the pre-incubation method to evaluated the contribution of formaldehyde to any bacterial mutagenicity detected. Formaldehyde was included as an additional positive control for strains TA98, TA100 and TA102 in the presence of S9.

Test articles as described in the report:

chemical	Lot or batch	Purity
Test article 1: clevidipine	2930.D.07.1	99.2% w/w
Test article 2: clevidipine +degradants	223-25	
Vial 1-5 *		
1237.9 mg clevidipine +	YH-223-10-C50	chromatographic purity
1237.9 mg clevidipine +	M08-0163-01	chromatographic purity
1237.9 mg clevidipine +	YH-223-18-III	chromatographic purity
Reference article formaldehyde Aqueous 37% w/v solution		

Purity of the degradants taken from the chemical analysis pages.
*The report did not specify what was contained in vial 1, vial 2, vial 3, vial 4, vial 5.

I was confused as to the disposition of the degradants based upon the following statement:

6.3. Test Article 2	
Identity	Clevidipine with degradants(API + Deg)
Lot number	223-25 (vials 1 to 5) containing a total of 1237.9 mg Clevidipine, degradants respectively, i.e. nominally each degradant
Preparation	Prepared by by dispensing weighed amounts of lot number 2930.D.07.1 plus individual degradants into individual vials. Detail of the composition of each vial is documented in raw data.

What was in which vial? Looking at the tables of results, it is not clear how the test articles (vials 1-5) were combined to achieve the concentrations.

The sponsor was contacted by email to provide clarification. The email is attached as Appendix I. The information contained in the reply is included here:

- For the Bacterial Mutation Test and Chromosome Aberration Test, each vial (Test Article 2) contained clevidipine with all three degradants (
- In anticipation of these studies being conducted by eight separate vials containing clevidipine with all three degradants were prepared by for reconstitution in DMSO by These vials were prospectively prepared to facilitate preparation of Test Article 2 solutions without their having to perform multiple weighings. Report number TMC-R307, Attachment 1 (attached, which was prepared by provides the weight of each material which was transferred into each vial. This report also provides the detailed directions which were used for their reconstitution. Following preparation, the 8 vials were shipped (on dry ice) to and stored at 2-6C (in the dark) until use.

- Of the 8 vials prepared by ——— individually reconstituted vials 1-5, and combined these solutions to create the single test solution referenced on page 40 of our submission (page 13 of the Bacterial Mutation Test Report).
- The Chromosome Aberration Test (please see page 112 of our submission, or page 12 of the Chromosome Aberration Test Report) used only the contents of vial number 6, which also contains clevidipine with all three degradants ———

——— have been indentified as degradation products in clevidipine drug product (clevidipine butyrate injectable emulsion) with proposed individual release and stability specifications of Not More Than (NMT) ——— This specification was the rationale for the concentration of each of ——— used in this assay.

Text Table 1 Study Design for Plate Incorporation and Pre-incubation Assays

Material	Formulation conc. (µg/mL)	Final conc. (µg/plate)	Plate incorporation		Pre-incubation		
			0S9	+S9	0S9	+S9	+S9 +FDH*
Vehicle	0	0	3	3	3	3	3
API	800	80	3	3	3	3	3
	1600	160	3	3	3	3	3
	3200	320	3	3	3	3	3
	6400	640	3	3	3	3	3
	12800	1280	3	3	3	3	3
	25600	2560	3	3	3	3	3
	50000	5000†	3	3	3	3	3
API+Deg	800	80	3	3	3	3	3
	1600	160	3	3	3	3	3
	3200	320	3	3	3	3	3
	6400	640	3	3	3	3	3
	12800	1280	3	3	3	3	3
	25600	2560	3	3	3	3	3
	50000	5000†	3	3	3	3	3
HCHO*	50	5				3	3
	100	10				3	3
	200	20				3	3
	400	40				3	3
Positive control	‡	‡	3	3	3	3	

‡ Depends on the test organism, positive control agent and methodology used
† Test article was tested at levels up to 5000 µg/plate, which is the standard limit dose recommended by regulatory guidelines
* With formaldehyde sensitive strains TA98, TA100 and TA102 only
API and API+Deg indicates Clevidipine without and with degradants, respectively

Plates were examined visually and in some cases with an inverted microscope. The sponsor provided criteria by which they assessed mutagenic potential and validity of the assay (copied from sponsor's report):

1. The results are considered positive (i.e. indicative of mutagenic potential) if both the following criteria are met.

• The results for the test article show a substantial increase in revertant colony counts, i.e. response = 2 times the concurrent vehicle control values for TA98, TA1535, TA1537 and WP2 uvrA, or = 1.5 times for TA100 and TA102, with mean value(s) outside the laboratory historical control range (beyond the 98% tolerance limit). Otherwise results are considered negative.

• The above increase must be dose related and/or reproducible i.e. increases must be obtained at more than one experimental point (more than one strain or dose level, more than one occasion or with different methodologies).

2. If the second criterion is not met, further testing might be appropriate to clarify equivocal results using an appropriately modified study design, e.g. a narrower dose interval with the appropriate strain. In such cases, if no substantial increase is obtained in the confirmatory test, the results would be considered negative.

For an assay to be considered valid, the mean revertant colony counts of the vehicle controls for each strain should lie close to or within the current historical control range of the laboratory. All positive control articles (with S9 mix where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5× for strain TA100 and TA102).

Results

Plate Incorporation Assay

The positive controls produced appropriate responses. Formaldehyde tested by itself produced a modest increase in the number of revertants.

Precipitate was reported for clevidipine concentrations ≥320 µg/plate and for clevidpine +degradants. This was reported not to interfere with evaluating the plates.

In the absence of S9, there was a slight increase in the number of revertants for strain TA1537 from concentrations of 160 µg/plate to 640 µg/plate.

Table 3 Clevidipine - Plate Incorporation Assay in the Absence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †	
			x_1	x_2	x_3	mean	SD	x_1	x_2	x_3		
TA1535	DMSO	0	14	20	12	**15**	4				1.0	
	80	0	25	13	17	**18**	6				1.2	
	160	0	25	25	22	**24**	2				1.6	
	320	0	24	26	20	**23**	3	ppt	ppt	ppt	1.5	
	640	0	23	12	22	**19**	6	ppt	ppt	ppt	1.2	
	1280	0	11	18	25	**18**	7	ppt	ppt	ppt	1.2	
	2560	0	14	13	18	**15**	3	ppt	ppt	ppt	1.0	
	5000	0	15	12	15	**14**	2	ppt	ppt	ppt	0.9	
TA1537	DMSO	0	6	6	3	**5**	2				1.0	
	80	0	6	6	10	**7**	2				1.5	
	160	0	13	6	13	**11**	4				2.1	B
	320	0	9	9	14	**11**	3	ppt	ppt	ppt	2.1	B
	640	0	13	14	8	**12**	3	ppt	ppt	ppt	2.3	B
	1280	0	8	11	7	**9**	2	ppt	ppt	ppt	1.7	
	2560	0	4	5	8	**6**	2	ppt	ppt	ppt	1.1	
	5000	0	7	7	9	**8**	1	ppt	ppt	ppt	1.5	
TA98	DMSO	0	22	22	25	**23**	2				1.0	
	80	0	15	20	20	**18**	3				0.8	
	160	0	22	12	23	**19**	6				0.8	
	320	0	22	13	24	**20**	6	ppt	ppt	ppt	0.9	
	640	0	14	13	11	**13**	2	ppt	ppt	ppt	0.6	
	1280	0	29	13	11	**18**	10	ppt	ppt	ppt	0.8	
	2560	0	13	22	15	**17**	5	ppt	ppt	ppt	0.7	
	5000	0	13	11	13	**12**	1	ppt	ppt	ppt	0.5	T

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation (note that SDs based on two values may be unreliable)

T If noted, toxic as indicated by low revertant colony counts (fold response < 0.6), or incomplete/ no background lawn (no meaningful count results for plates with IL or NL)

\+ If noted, substantial dose-related increase in revertant colony counts (fold response ≥ 2.0 or ≥ 1.5 for TA100 and TA102)

When degradants were added to clevidipine, there was no discernible change in the revertant frequency for TA1537.

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ON ORIGINAL

Table 5 Clev. + deg - Plate Incorporation Assay in the Absence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †	
			x_1	x_2	x_3	**mean**	SD	x_1	x_2	x_3		
TA1535	DMSO	0	14	20	12	**15**	4				1.0	
	80	0	31	22	17	**23**	7				1.5	
	160	0	24	15	26	**22**	6				1.4	
	320	0	24	22	23	**23**	1	ppt	ppt	ppt	1.5	
	640	0	23	15	21	**20**	4	ppt	ppt	ppt	1.3	
	1280	0	12	13	12	**12**	1	ppt	ppt	ppt	0.8	
	2560	0	17	17	13	**16**	2	ppt	ppt	ppt	1.0	
	5000	0	20	9	19	**16**	6	ppt	ppt	ppt	1.0	
TA1537	DMSO	0	6	6	3	**5**	2				1.0	
	80	0	10	16	7	**11**	5				2.2	B
	160	0	8	12	17	**12**	5				2.5	B
	320	0	9	9	15	**11**	3	ppt	ppt	ppt	2.2	B
	640	0	7	11	8	**9**	2	ppt	ppt	ppt	1.7	
	1280	0	12	11	13	**12**	1	ppt	ppt	ppt	2.4	B
	2560	0	6	8	12	**9**	3	ppt	ppt	ppt	1.7	
	5000	0	4	4	3	**4**	1	ppt	ppt	ppt	0.7	

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation (note that SDs based on two values may be unreliable)

T If noted, toxic as indicated by low revertant colony counts (fold response < 0.6), or incomplete/ no background lawn (no meaningful count results for plates with IL or NL)

\+ If noted, substantial dose-related increase in revertant colony counts (fold response ≥ 2.0 or ≥ 1.5 for TA100 and TA102)

A Substantial increase in revertant colony counts (fold response ≥ 2.0) probably due to normal variation than dose related.

B Apparent increase considered to be due to somewhat low concurrent vehicle counts since not dose-related and not outside historical control range

In the presence of S9, TA98, showed concentration related increases in numbers of revertants.

Table 4 Clevidipine - Plate Incorporation Assay in the Presence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †	
			x_1	x_2	x_3	**mean**	SD	x_1	x_2	x_3		
TA98	DMSO	+	28	41	26	**32**	8				1.0	
	160	+	22	31	28	**27**	5				0.9	
	320	+	26	22	25	**24**	2				0.8	
	640	+	34	32	28	**31**	3				1.0	
	1280	+	50	55	55	**53**	3				1.7	
	2560	+	62	79	106	**82**	22				2.6	+
	5000	+	37	23	42	**34**	10	ppt	ppt	ppt	1.1	

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)
† Fold response in mean revertants compared to concurrent vehicle control
SD Sample standard deviation (note that SDs based on two values may be unreliable)
T If noted, toxic as indicated by low revertant colony counts (fold response < 0.6), or incomplete/ no background lawn (no meaningful count results for plates with IL or NL)
\+ If noted, substantial dose-related increase in revertant colony counts (fold response ≥ 2.0 or ≥ 1.5 for TA100 and TA102)
A Substantial increase in revertant colony counts (fold response ≥ 2.0) probably due to normal variation than dose related.
B Apparent increase considered to be due to somewhat low concurrent vehicle counts since not dose-related and not outside historical control range

Addition of degradants did not appreciably change the number of revertants.

Table 6 Clev. + deg - Plate Incorporation Assay in the Presence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †	
			x_1	x_2	x_3	**mean**	SD	x_1	x_2	x_3		
TA98	DMSO	+	28	41	26	**32**	8				1.0	
	80	+	31	30	31	**31**	1				1.0	
	160	+	30	28	33	**30**	3				1.0	
	320	+	35	29	39	**34**	5				1.1	
	640	+	36	46	37	**40**	6				1.3	
	1280	+	50	58	46	**51**	6				1.6	
	2560	+	109	75	89	**91**	17				2.9	+
	5000	+	39	24	33	**32**	8	ppt	ppt	ppt	1.0	

Strains TA100 and TA102 in the presence of S9 also showed a concentration-related increase in numbers of revertants.

Table 4 Clevidipine - Plate Incorporation Assay in the Presence of S9 Mix (Cont'd)

Strain	Conc. (µg/plate)	S9	Number of revertants x_1	x_2	x_3	mean	SD	Plate observations * x_1	x_2	x_3	Fold response †	
TA100	DMSO	+	132	148	132	**137**	9				1.0	
	160	+	168	168	156	**164**	7				1.2	
	320	+	162	181	163	**169**	11				1.2	
	640	+	178	159	162	**166**	10				1.2	
	1280	+	223	173	202	**199**	25				1.5	+
	2560	+	212	232	251	**232**	20				1.7	+
	5000	+	55	87	103	**82**	24	ppt	ppt	ppt	0.6	
TA102	DMSO	+	559	487	609	**552**	61				1.0	
	160	+	520	579	607	**569**	44				1.0	
	320	+	492	607	631	**577**	74				1.0	
	640	+	647	666	709	**674**	32				1.2	
	1280	+	843	902	924	**890**	42				1.6	+
	2560	+	1386	1191	1049	**1209**	169				2.2	+
	5000	+	1463	924	964	**1117**	300	ppt	ppt	ppt	2.0	+

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation (note that SDs based on two values may be unreliable)

T If noted, toxic as indicated by low revertant colony counts (fold response < 0.6), or incomplete/ no background lawn (no meaningful count results for plates with IL or NL)

\+ If noted, substantial dose-related increase in revertant colony counts (fold response ≥ 2.0 or ≥ 1.5 for TA100 and TA102)

A Substantial increase in revertant colony counts (fold response ≥ 2.0) probably due to normal variation than dose related.

B Apparent increase considered to be due to somewhat low concurrent vehicle counts since not dose-related and not outside historical control range

The addition of the degradants did not significantly change the revertant incidence.

Pre-Incubation Assay

Precipitate was again reported for concentrations ≥320 μg/plate.

Without S9, there was a doubling of the reverant rate in strain TA1537 at one concentration (640 μg/plate). Without S9, strains TA98, TA100 and TA102 did not show any change in revertant frequency when treated with clevidipine.

In the presence of S9, TA98, TA100 and TA102 again showed concentration related increases in revertant frequency consistent with the results of the plate incorporation assay. When degradants were added along with the clevidipine, the frequency of revertants did not change significantly.

Pre-Incubation Assay + FDH
The addition of FDH to the assay mix caused a decrease in the number of revertants reported in all 3 strains, but did not completely abolish them in TA98 and TA100. The fold increases in revertants produced without FDH is shown also for comparison.

Summary of the pre-incubation assay for 3 bacterial strains +S9

	TA98 fold response		TA100 fold response		TA102 fold response	
Conc µg/plate	Clevidipine only	Clevidipine + degradants	Clevidipine only	Clevidipine + degradants	Clevidipine only	Clevidipine + degradants
DMSO	1.0	1.0	1.0	1.0	1.0	1.0
80	1.1	1.6	1.4	1.5	0.8	1.0
160	2.6	2.3	1.4	1.9	1.0	1.1
320	5.0	4.8	2.2	3.0	1.5	1.8
640	4.2	4.0	3.2	4.1	2.8	2.9
1280	1.8	2.3	1.4	1.5, IL,T	1.7	2.4
2560	IL, T	IL, T	IL,T	IL, T	2.0	2.2
5000	Ppt, IL, T	Ppt, IL, T	ppt, IL, T	Ppt NL, T	Ppt, IL, T	Ppt IL, T
With FDH						
DMSO	1.0	1.0	1.0	1.0	1.0	1.0
80	1.1	0.9	1.0	1.0	1.0	1.0
160	1.6	1.3	1.0	1.0	1.1	1.0
320	3.2	2.7	1.1	1.1	1.2	1.2
640	4.1	3.8	2.2	2.4	1.6	1.4
1280	2.1	2.3	1.7	IL, T	1.3	1.2
2560	IL, T	0.9	IL, T	IL, T	0.7	1.0
5000	Ppt IL, T	Ppt, IL, T	Ppt, NL,T	Ppt IL, T	Ppt, IL, T	Ppt, IL, T

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)
† Fold response in mean revertants compared to concurrent vehicle control
SD Sample standard deviation (note that SDs based on two values may be unreliable)
T If noted, toxic as indicated by low revertant colony counts (fold response < 0.6), or incomplete/ no background lawn (no meaningful count results for plates with IL or NL)
\+ If noted, substantial dose-related increase in revertant colony counts (fold response ≥ 2.0 or ≥ 1.5 for TA100 and TA102)
A Substantial increase in revertant colony counts (fold response ≥ 2.0) probably due to normal variation than dose related.
B Apparent increase considered to be due to somewhat low concurrent vehicle counts since not dose-related and not outside historical control range

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The data for formaldehyde, a positive control, is also shown for comparison.

Table 12 Formaldehyde - Pre incubation Assay in the Presence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †	
			x_1	x_2	x_3	mean	SD	x_1	x_2	X_3		
TA98	Water	+	24	36	26	**29**	6				1.0	
	5	+	33	44	50	**42**	9				1.5	
	10	+	58	65	38	**54**	14				1.9	
	20	+	108	116	101	**108**	8				3.8	+
	40	+	104	96	60	**87**	23				3.0	+
TA100	Water	+	119	167	168	**151**	28				1.0	
	5	+	198	230	220	**216**	16				1.4	
	10	+	324	283	305	**304**	21				2.0	+
	20	+	426	371	497	**431**	63				2.9	+
	40	+	282	210	258	**250**	37				1.7	+
TA102	Water	+	706	781	686	**724**	50				1.0	
	5	+	686	711	732	**710**	23				1.0	
	10	+	958	937	1016	**970**	41				1.3	
	20	+	841	757	856	**818**	53				1.1	
	40	+	1355	1212	1206	**1258**	84				1.7	+

Summary

Plate incorporation assay: Under the conditions of the assay, clevidipine by itself caused a slight increase in the number of revertants for strain TA1537 in the absence of S9, from concentrations of 160µg/plate to 640 µg/plate. Above that level, precipitate may have affected the ability to evaluate the plate. With S9, strains TA98, TA100 and TA102 each showed concentration related increases in the numbers of revertants. There was no discernible change in the numbers of revertants when the degradants and clevidpine were added together to the culture plates.

Pre-incubation assay: Under the conditions of the assay, the results were consistent with those of the plate incorporation assay. Strain TA1537 showed a slight increase in revertant frequency without S9. With S9, strains TA98, TA100 and TA102 each showed concentration related increases in revertant frequency. There was no discernible change in the numbers of revertants when the degradants and clevidpine were added together to the culture plates.

Pre-incubation assay with FDH: The addition of FDH did not completely abolish the increase in revertants seen in the cultures of strains TA98, TA102 and TA100.

The sponsor states that concentrations of FDH have been optimized for the different Salmonella strains based on previous work. Some variability in the assay systems are to be expected. Was

the lack of total abrogation of the increase in revertants due to some genotoxic capacity other than the generation of formaldehyde? Does the site of generation of the formaldehyde influence these results? That is, if clevidipine is degraded in the extracellular medium where the FDH can access the formaldehyde that is generated, is the response greater than if the clevidipine is internalized and degraded intracellularly? What was demonstrated in this study is that the addition of the 3 degradants in concentrations of — each to clevidpine did not discernibly alter the frequency of revertants in the cultures of bacterial strains used. The addition of FDH to the assay systems decreased but did not totally abolish the increase in revertants.

Clevidipine with and without Degradants* — *Chromosome Aberration Test

Study location: —

Report number: 962194
GLP: statement included
QA: yes
Study dates: experimental phase from April 9,2008 – May 5, 2008
Vehicle: DMSO
Cells used: primary cultures of human peripheral lymphocytes collected from healthy non-smoking male donors
S9 mix: from male SD rats induced with Phenobarbital/ 5,6-benzoflavone (obtained from —

Positive controls: cyclophosphamide
Mitomycin C

Test article	Batch or lot #	Purity
Clevidpine butyrate	2930.D.07.1	99.2% by HPLC
Test article 2: clevidipine + degradants (see sponsor's paragraph below)		
Degradant —	YH-223-10-C50	— hromatographic
Degradant —	M08-0163-01	chromatographic
Degradant —	YH-223-18-III	chromatographic

6.3. Test Article 2

Identity	Clevidipine with degradants (API+Deg)
Preparation	Prepared by — by dispensing weighed amounts of lot number 2930.D.07.1 plus individual degradants into individual vials.
Lot number	223-25-6 (vial number 6) containing 248.40 mg Clevidipine, — degradants — respectively, i.e. nominally — each degradant

The sponsor was requested to clarify the methodology used for the combination of clevidipine and degradants. The sponsor's answer is reproduced below:

- For the Bacterial Mutation Test and Chromosome Aberration Test, each vial (Test Article 2) contained clevidipine with all three degradants
- In anticipation of these studies being conducted by eight separate vials containing clevidipine with all three degradants were prepared by for reconstitution in DMSO by these vials were prospectively prepared to facilitate preparation of Test Article 2 solutions without their having to perform multiple weighings. Report number TMC-R307, Attachment 1 (attached, which was prepared by provides the weight of each material which was transferred into each vial. This report also provides the detailed directions which were used for their reconstitution. Following preparation, the 8 vials were shipped (on dry ice) to and stored at 2-6C (in the dark) until use.
- Of the 8 vials prepared by individually reconstituted vials 1-5, and combined these solutions to create the single test solution referenced on page 40 of our submission (page 13 of the Bacterial Mutation Test Report).
- The Chromosome Aberration Test (please see page 112 of our submission, or page 12 of the Chromosome Aberration Test Report) used only the contents of vial number 6, which also contains clevidipine with all three degradants

have been identified as degradation products in clevidipine drug product (clevidipine injectable emulsion) with proposed individual release and stability specifications of Not More Than (NMT . This was the rationale for the choice of concentration of each degradant used in this assay.

Human peripheral blood lymphocytes were stimulated to divide prior to treatment. All treatments were performed approximately 48 hours after culture initiation. A standard dose volume of 10 µl vehicle/test article/positive control per ml culture was used throughout the study. Duplicate cultures were treated at each experimental point. The highest concentration used was one expected to show definite genotoxicity in the presence of S9 based on previous work.

Text Table 1 Study Design - Vehicle and Test Articles

Material	Concentration			Number of Culture 0FDH			Number of Culture +FDH		
	Form. µg/mL	Final µg/mL	Final µM	4Hr 0S9	4Hr +S9	21Hr 0S9	4Hr 0S9	4Hr +S9	21Hr 0S9
Vehicle	-	-		2	2	2	2	2	2
API	3650	36.5	80	2	2	2	2	2	2
	5162	51.6	113	2	2	2	2	2	2
	7301	73.0	160	2	2	2	2	2	2
	10325	103.3	226	2	2	2	2	2	2
	14602	146.0	320	2	2	2	2	2	2
	20650	206.5	453	2	2	2	2	2	2
API+Deg	3650	36.5	80	2	2	2	2	2	2
	5162	51.6	113	2	2	2	2	2	2
	7301	73.0	160	2	2	2	2	2	2
	10325	103.3	226	2	2	2	2	2	2
	14602	146.0	320	2	2	2	2	2	2
	20650	206.5	453	2	2	2	2	2	2

Text Table 2 Study Design - Positive controls

Material	Concentration		Number of Culture 0FDH			Number of Culture +FDH		
	Form. µg/mL	Final µg/mL	4Hr 0S9	4Hr +S9	21Hr 0S9	4Hr 0S9	4Hr +S9	21Hr 0S9
MMC	5.0	0.05	2	-	2			
	10.0	0.10	2	-	2			
	20.0	0.20	2	-	2			
CP	800	8.0	-	2	-			
	1200	12.0	-	2	-			
	1600	16.0	-	2	-			
HCHO	188	1.88	2	2	2	2	2	2
	750	7.50	2	2	2	2	2	2
	3000	30.0	2	2	2	2	2	2

MMC = Mitomycin C, CP = cyclophosphamide, HCHO = formaldehyde, Form. = formulation
API and API+Deg indicates Clevidipine without and with degradants, respectively

After the 4-hour treatment, the appropriate cultures were centrifuged, the supernatant discarded and replaced with fresh medium. The cultures were then returned to the incubator for another 17 hours incubation before metaphase harvesting.

Colcemid was added to all cultures approximately 2 hours prior to harvesting. At least two slides per culture were washed with 3 changes of purified water and stained with Giemsa solution. The mitotic index was determined by evaluation of at least 500 cells per culture for selected treatment groups, defined as dose levels not showing extreme toxic effects. Relative mitotic index (RMI) was calculated as a percentage ratio compared with the appropriate concurrent vehicle control group.

Slides for examination of aberrations were selected primarily based on the RMI results. For each treatment regime and phase, the highest dose level selected for examination of aberrations was the highest dose tested showing relatively low toxicity. Otherwise the highest level examined was the lowest concentration which caused a decrease in RMI to ≤50%. On occasion the treatment procedure may have a clear effect on the quality of metaphases or cause an extreme reduction in the absolute number of metaphases, in which case a different maximal dose for examination may be justified. At least two additional lower dose levels of the test article are assessed. The selected slides were encoded prior to evaluation to minimize potential operator bias. A total of 200 metaphases were examined for aberrations (1 slide per culture).

The sponsor also provided the criteria for assessment of the results:

> A positive response is normally indicated by a statistically significant (dose-related, if applicable) increase in the incidence of aberrant cells for the treatment group compared with the concurrent control group ($p \leq 0.01$); individual and/or group mean values should exceed the laboratory historical control range (99% limit).
>
> A negative result is indicated where group mean incidences of aberrant metaphase cells for the group treated with the test article are not significantly greater than incidences for the concurrent control group ($p > 0.01$) and where these values fall within or close to the historical control range.
> An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response.
>
> For an assay to be considered valid, the vehicle/negative control results should lie within or close to the historical control range, while the positive control should produce a significant increase in the incidence of aberrant cells compared with the concurrent control.

Results

The positive controls produced appropriate results.

Positive results were reported for the test article for all conditions tested. The sponsor's description is shown below.

12.3. Chromosome Aberrations

Cultures treated with Clevidipine without degradants showed dose-related and statistically significant increases in the incidence of cells with aberrant metaphases for all treatment regimes, i.e. short and long treatments in the absence of S9 and the short treatment in the presence of S9. Equivalent results were obtained for Clevidipine with degradants. In the presence of formaldehyde dehydrogenase (FDH), there was a slight apparent reduction in the response which could be due to detoxification of formaldehyde produced by release from Clevidipine. However, no clear reduction in response to formaldehyde (when added directly in bulk to cultures) was seen with FDH in this study, so the effectiveness of FDH in detoxifying formaldehyde could not be confirmed in this system (see Table 1 for summary and Appendix 2 for detailed results). In addition, the proportion of aberrant metaphases for all vehicle control groups was within the laboratory historical control range (see Figure 1 for negative and positive historical control results).

As may be seen in the sponsor's data tables, the test article produced a dose response of increased aberrations. There were no significant differences when degradants and clevidipine were added to the test mixture. There was a slight decrease in the number of aberrations when FDH was added to the cultures, but statistically significant increases were still reported. In some cases the response of the test article exceeds that of the positive control. The values also exceed the range of historical control values.

Table 1 Results and Statistical Analysis

Treatment	Final Concentration (µg/mL)	Final Concentration (µM)	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations b	e	B	E	other	Incidental observations † (g	G	P	C)
4 hours treatment in the absence of S9 (0S9) in absence of FDH															
DMSO	-	-	8.4	100	200	2.0	4	0	0	0	0	3	0	0	1
API	103.2	226	8.8	106	200	4.5	5	0	4	0	0	1	0	3	0
	146.0	320	6.3	75	200	12.0**	17	1	9	0	0	9	3	6	0
	206.5	453	3.5	42	200	22.5**	55	4	4	0	0	7	0	2	2
MMC	0.10	-	7.2	87	200	9.0**	16	3	1	0	0	6	0	0	0
DMSO	-	-	10.8	100	200	2.0	3	0	1	0	0	0	0	0	0
API-Deg	103.2	226	9.7	90	200	0.5	0	0	1	0	0	0	0	2	1
	146.0	320	10.0	93	200	12.0**	18	5	8	0	0	0	0	10	0
	206.5	453	7.8	73	200	16.0**	21	12	13	0	0	0	0	8	0
HCHO		-	8.8	82	200	12.5**	13	16	10	0	0	2	0	5	2
4 hours treatment in the absence of S9 (0S9) in presence of FDH															
DMSO	-	-	9.9	100	200	0.5	1	0	0	0	0	1	0	0	0
API	103.2	226	9.4	95	200	5.0*	9	1	2	0	0	0	0	4	0
	146.0	320	5.5	56	200	9.0**	12	2	5	0	0	5	0	10	0
	206.5	453	6.1	62	200	16.5**	29	4	7	0	0	3	0	4	1
DMSO	-	-	9.8	100	200	0.0	0	0	0	0	0	0	0	0	1
API-Deg	103.2	226	9.7	99	200	0.5	1	0	0	0	0	1	0	4	0
	146.0	320	7.9	80	200	7.0**	9	2	4	0	0	0	0	5	0
	206.5	453	4.7	48	200	15.0**	14	8	19	0	0	3	0	3	0
HCHO	7.50	-	7.6	77	200	8.0*	6	7	7	0	0	1	0	2	0

MI, RMI Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g Chromatid break, exchange, gap
B, E, G Chromosome break, exchange, gap
other Includes pulverized chromosomes and cells with > 8 aberrations
P Polyploidy and endoreduplication
C Centromeric disruption
† g, G, P and C are excluded from the calculation of % aberrant cells
Results of statistical analysis using one-tailed Fisher's exact test
* $p \leq 0.01$ (significant)
** $p \leq 0.001$ (highly significant)
otherwise $p > 0.01$ (not significant)

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Table 1 Results and Statistical Analysis (Cont'd)

Treatment	Final Concentration (µg/mL)	Final Concentration (µM)	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations b	e	B	E	other	Incidental observations † (g	G	P	C)
4 hours treatment in the presence of S9 (+S9) in absence of FDH															
DMSO	-	-	6.4	100	200	1.0	1	0	1	0	0	2	0	1	2
API	103.2	226	4.3	67	200	6.0*	12	0	0	0	0	3	0	2	1
	146.0	320	10.0	155	200	15.0**	29	10	8	0	0	4	0	10	0
	206.5	453	4.2	66	200	27.0**	61	11	5	0	0	17	3	2	0
CP	8.0	-	3.1	48	200	24.5**	47	8	7	0	0	13	2	0	0
DMSO	-	-	7.1	100	200	0.0	0	0	0	0	0	1	1	0	1
API-Deg	103.2	226	8.8	125	200	2.0	2	0	2	0	0	1	0	4	0
	146.0	320	9.1	129	200	11.0**	13	3	15	0	0	0	1	2	1
	206.5	453	3.7	52	200	18.5**	22	9	18	0	0	5	1	3	1
HCHO	1.88	-	12.1	171	200	5.0**	5	1	5	0	0	0	0	2	0
4 hours treatment in the presence of S9 (+S9) in presence of FDH															
DMSO	-	-	6.7	100	200	0.0	0	0	0	0	0	1	0	0	1
API	103.2	226	6.6	97	200	6.0**	10	1	1	0	0	4	0	6	1
	146.0	320	6.0	89	200	11.0**	22	9	0	0	0	10	1	4	1
	206.5	453	4.7	70	200	17.5**	34	12	1	0	0	4	0	1	0
DMSO	-	-	5.8	100	200	0.0	0	0	0	0	0	0	0	0	0
API-Deg	103.2	226	7.4	128	200	5.5**	6	2	4	0	0	0	0	4	0
	146.0	320	5.8	100	200	6.0**	5	4	6	0	0	0	0	5	2
	206.5	453	4.0	69	200	15.0**	20	5	9	0	0	0	0	3	1
HCHO	7.50	-	7.3	68	200	7.5**	5	9	6	1	0	0	1	7	2

MI, RMI Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g Chromatid break, exchange, gap
B, E, G Chromosome break, exchange, gap
other Includes pulverized chromosomes and cells with > 8 aberrations
P Polyploidy and endoreduplication
C Centromeric disruption
† g, G, P and C are excluded from the calculation of % aberrant cells
Results of statistical analysis using one-tailed Fisher's exact test
* $p \leq 0.01$ (significant)
** $p \leq 0.001$ (highly significant)
otherwise $p > 0.01$ (not significant)

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Table 1 Results and Statistical Analysis (Cont'd)

Treatment	Final Concentration (µg/mL)	Final Concentration (µM)	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations b	e	B	E	other	Incidental observations † (g	G	P	C)
21 hours treatment in the absence of S9 (0S9) in absence of FDH															
DMSO	-	-	6.7	100	200	0.0	0	0	0	0	0	1	0	3	0
API	103.2	226	11.3	169	200	4.5*	8	0	1	0	0	0	0	0	0
	146.0	320	5.6	83	200	9.5**	16	1	2	0	0	8	0	1	0
	206.5	453	3.2	48	200	19.5**	51	1	13	0	0	6	0	0	2
MMC	0.05	-	4.3	64	200	4.5*	7	2	1	0	0	9	0	0	1
DMSO	-	-	6.6	100	200	0.0	0	0	0	0	0	0	0	0	0
API-Deg	103.2	226	13.8	210	200	3.5*	4	0	3	0	0	0	0	2	0
	146.0	320	8.8	134	200	11.0**	10	1	14	0	0	1	0	4	1
	206.5	453	2.0	30	200	11.5**	12	3	17	0	2	3	0	0	1
HCHO	7.50	-	6.0	91	200	10.0**	9	9	4	0	0	0	0	5	0
21 hours treatment in the absence of S9 (0S9) in presence of FDH															
DMSO	-	-	5.5	100	200	1.5	4	0	0	0	0	3	0	0	1
API	73.0	160	5.1	92	200	1.5	2	0	1	0	0	5	0	1	0
	103.2	226	11.3	205	200	3.0	4	1	1	0	0	0	0	3	1
	146.0	320	10.3	187	200	8.5*	19	1	0	0	0	3	1	7	0
	206.5	453	1.8	33	(127	14.2	28	0	7	0	0	6	1	0	1)
DMSO	-	-	6.9	100	200	0.0	0	0	0	0	0	0	0	1	0
API-Deg	103.2	226	12.1	176	200	2.0	2	0	3	0	0	1	0	1	0
	146.0	320	8.3	121	200	8.5**	7	3	7	0	0	0	0	6	0
	206.5	453	1.6	23	200	12.0**	21	0	10	0	1	4	0	0	2
HCHO	7.50	-	6.2	91	200	8.5**	13	0	8	0	0	1	0	2	0

MI, RMI Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g Chromatid break, exchange, gap
B, E, G Chromosome break, exchange, gap
other Includes pulverized chromosomes and cells with > 8 aberrations
P Polyploidy and endoreduplication
C Centromeric disruption
† g, G, P and C are excluded from the calculation of % aberrant cells
For results in brackets, no statistical analysis was performed
Results of statistical analysis using one-tailed Fisher's exact test
* $p \leq 0.01$ (significant)
** $p \leq 0.001$ (highly significant)
otherwise $p > 0.01$ (not significant)

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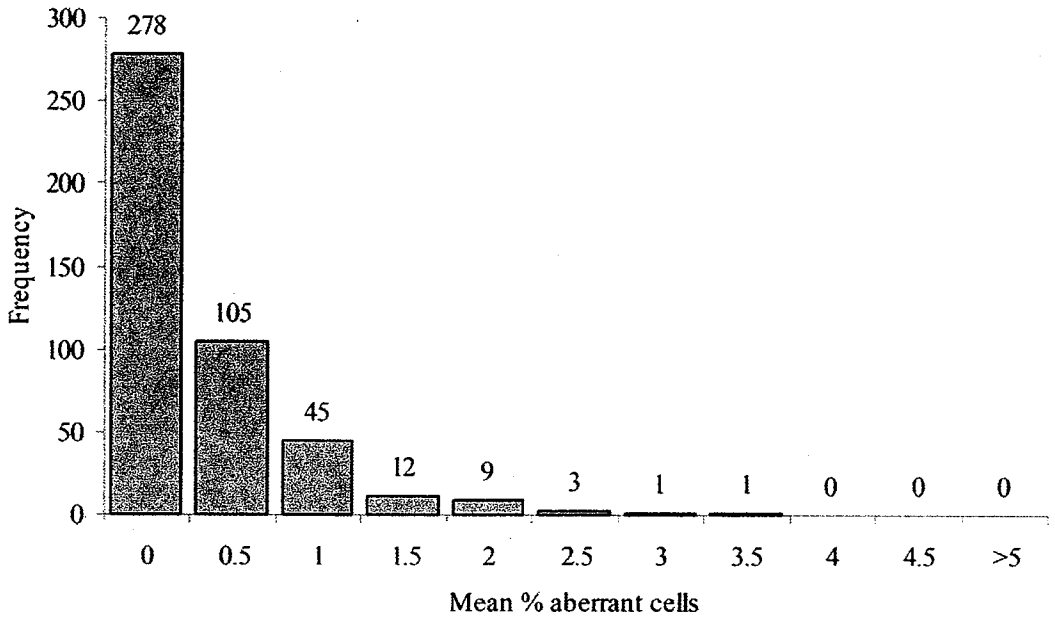
Figure 1 **Historical Control Values**



The laboratory historical mean incidence of aberrant metaphase cells for negative/vehicle control cultures for the human lymphocyte chromosome aberration test is 0.32% (SD 0.53) for 454 treatments. These QA audited results were collected from GLP compliant studies performed from 05 February 2003 to 15 November 2007.

The historical positive control values (for QA-audited and GLP compliant studies) are listed below:

Mitomycin C (4 hour 0S9):	mean 10.4%, SD 5.1, 112 treatments
Mitomycin C (21 hour 0S9):	mean 12.0%, SD 5.1, 116 treatments
Cyclophosphamide (4 hour +S9):	mean 19.5%, SD 7.6, 115 treatments

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Study T3376 " Analysis of structural chromosome aberrations in human lymphocytes treated with clevidipine (H324/38) in vitro" was conducted in 1996 and was cited in the NDA review. Some of the results of that earlier study are included here for purposes of comparison and completeness. Without metabolic activation, FDH apparently eliminated the chromosomal aberrations

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TABLE 1

THE EFFECT OF H324/38 WITHOUT METABOLIC ACTIVATION ON THE FREQUENCY OF CHROMOSOME ABNORMALITIES IN HUMAN LYMPHOCYTES IN VITRO

Concentration mmol/l	No. of meta-phases	Abnormal metaphases %	Number of aberrations: Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	M Abs[a]	No. of gaps	Mitoses /1000 cells
First harvest									
Medium control	200	2.0	4	0	0	0	0	2	32
Solvent control	200	1.5	3	0	0	0	0	8	31
0.080	200	1.0	1	0	1	0	0	1	33
0.16	200	3.0	3	0	3	0	0	5	37
0.32	200	8.0**	12	1	4	0	0	8	79
Positive control, MMS[b] 0.22	200	41***	67	23	12	0	0	23	36
Second harvest									
Solvent control	200	1.5	1	0	1	1	0	1	16
0.16	200	4.0	7	0	2	0	0	3	66

** = 0.01>P>0.001
*** = P<0.001
a = Multiple aberrations
b = Methyl methanesulfonate

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21(47)

TABLE 3

THE EFFECT OF H324/38 AND FORMALDEHYDE DEHYDROGENASE (FDH[b]) WITHOUT METABOLIC ACTIVATION ON THE FREQUENCY OF CHROMOSOME ABNORMALITIES IN HUMAN LYMPHOCYTES IN VITRO

Concentration mmol/l	No. of meta-phases	Abnormal metaphases %	Number of aberrations: Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	M Abs[a]	No. of gaps	Mitoses /1000 cells
First harvest									
Medium control	200	1.0	1	0	0	1	0	3	22
Solvent control + FDH[b]	200	1.5	0	0	3	0	0	2	23
0.10 + FDH[b]	200	1.0	2	0	0	0	0	3	19
0.20 + FDH[b]	200	1.0	1	0	2	0	0	3	42
0.30 + FDH[b]	200	2.0	2	1	1	0	0	1	111
Positive control, MMS[c] 0.22	200	35***	53	35	3	0	0	34	30

** = 0.01>P>0.001
*** = P<0.001
a = Multiple aberrations
b = Formaldehyde dehydrogenase
c = Methyl methanesulfonate

In the presence of metabolic activation, FDH again apparently eliminated the chromosomal aberrations.

ASTRA

20(47)

TABLE 2

THE EFFECT OF H324/38 WITH METABOLIC ACTIVATION ON THE FREQUENCY OF CHROMOSOME ABNORMALITIES IN HUMAN LYMPHOCYTES IN VITRO

Concentration mmol/l	No. of meta-phases	Abnormal metaphases %	Number of aberrations: Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	M Abs[a]	No. of gaps	Mitoses /1000 cells
First harvest									
Solvent control	200	3.0	4	0	1	1	0	4	43
0.16	200	6.0	11	0	2	0	0	4	35
0.32	200	27***	44	10	16	0	0	20	25
0.40	200	40***	87	21	8	0	0	32	25
Second harvest									
Solvent control	200	2.0	3	0	1	0	0	3	36
0.40	200	6.5*	5	1	7	0	1	5	41
Positive control, CP[b] 0.060	200	21***	15	9	28	4	0	3	27

* = 0.05>P>0.01
*** = P<0.001
a = Multiple aberrations
b = Cyclophosphamide

ASTRA 22(47)

TABLE 4

THE EFFECT OF H324/38 AND FORMALDEHYDE DEHYDROGENASE (FDH[b]) WITH METABOLIC ACTIVATION ON THE FREQUENCY OF CHROMOSOME ABNORMALITIES IN HUMAN LYMPHOCYTES IN VITRO

Concentration mmol/l	No. of meta-phases	Abnormal metaphases %	Number of aberrations: Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	M Abs[a]	No. of gaps	Mitoses /1000 cells
First harvest									
Solvent control + FDH[b]	200	0	0	0	0	0	0	3	40
0.10 + FDH[b]	200	1.5	3	0	0	0	0	3	28
0.30 + FDH[b]	200	2.0	3	0	1	0	0	4	29
0.40 + FDH[b]	200	1.0	1	0	0	1	0	5	16
Positive control, CP[c] 0.030	200	51***	103	18	19	0	2	45	20

* = 0.05>P>0.01
*** = P<0.001
a = Multiple aberrations
b = Formaldehyde dehydrogenase
c = Cyclophosphamide

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Summary

There is consistency in these two chromosomal aberration studies performed 12 years apart. Given the normal intra-laboratory variability, inter-laboratory variability and a significant time span, perhaps the assays are as consistent as one could reasonably expect.

As originally demonstrated, clevidipine ±S9 caused a statistically significant increase in chromosomal aberrations. However, unlike the original study, the addition of FDH diminished but did not eliminate the increase in aberrations. The combination of degradants did not increase the incidence of revertants.

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Overall Summary and Conclusion

The material submitted was in response to the CMC and Pharmacology/Toxicology Discipline reviews. The requests made by the Disciplines were for 1) a receptor binding profile of clevidipine butyrate and its major metabolite H152/81 and 2) characterization of the potential genotoxicity of the degradants

Receptor screening study for clevidipine and its primary metabolite H152/81(M1)
Both clevidipine and H152/81 were tested at concentrations of 1nM, 100 nM and 10 μM in a standard receptor screening assay.

Clevidipine showed a significant level of binding to the L-type calcium channel receptor, the adenosine A2A receptor and the thromboxane A2 receptor. The degree of binding is summarized in the reviewer's table below. Negative inhibition of binding was unexplained.

Reviewer's summary of percent inhibition of known ligand binding by celvidipine

receptor	Concentration of clevidipine		
	1 nM	100 nM	10 μM
Adenosine A2A (h)	-16%	-9%	65%
L-type calcium channel	35%	74%	107%
Thromboxane A2	-12%	9%	84%

Clevidipine's main metabolite, H152/81, also showed a significant level of binding to the L-type calcium channel and to the thromboxane A2 receptor. The degree of binding is summarized in the reviewer's table below.

Summary of percent inhibition of known ligand binding by H152/81

receptor	Concentration of H152/81		
	1 nM	100 nM	10 μM
L-type calcium channel	18%	19%	87%
Thromboxane A2	8%	1%	85%

The sponsor provided a comparison of human blood values in this report which was reproduced in this review. The concentrations at which the thromboxane A2 receptor binding effects were reported fall within the sponsor's human concentration ranges (50-100 nM for clevidipine and 10-30 μM for H152/81). While it is difficult to translate in vitro concentration effects into in vivo effects, the sponsor did re-examine the rat and dog toxicology studies for signs that might be attributable to interactions with the thromboxane A2 receptor. That is, changes in hemostasis, thromboembolic phenomena, platelet aggregation, and bronchoconstriction. No events were

reported to suggest a significant in vivo effect of the TBXA2 receptor binding by clevidipine or the major metabolite.

Clevidipine with and without degradants ——— : Bacterial Mutation Test

Clevidipine butyrate was tested in the Ames bacterial reverse mutation assay ± a combination of degradants ——— have been identified as degradation products in clevidipine drug product (clevidipine butyrate injectable emulsion) with proposed individual release and stability specifications of Not More Than (NMT) — Based upon these specifications, the sponsor added each degradant to the assay at a concentration of —— The Ames assay was also conducted ± formaldehyde dehydrogenase to assess the genotoxic contributions of formaldehyde generated from clevidipine. The study as reported was adequate.

In both the plate incorporation assay and the pre-incubation assay, without S9, there was a slight increase in the number of revertants (2-2.5 fold increase over the vehicle control incidence) reported for Salmonella strain TA1537 from concentrations of 160μg/plate to 640 μg/plate. When degradants were added to the cultures, there was no appreciable change in the incidence of revertants.

In both assays, in the presence of S9, TA98, TA100 and TA102 showed concentration related increases in revertants. Addition of degradants did not discernibly change the revertant frequency. For each of these strains, the increase in revertants was ≤3 fold the vehicle frequency.

Formaldehyde dehydrogenase was used in the pre-incubation assay. The addition of FDH to the assay mix caused a decrease in the number of revertants reported but did not completely abolish them in all 3 strains. This suggests that there may be differences in strain sensitivity, differences in strain metabolism, some other genotoxicant or simply a degree of variability inherent in the assay.

Under the conditions of the assays, the degradants did not show additive or synergistic genotoxic potential when tested in combination with clevidipine.

Clevidipine with and without Degradants ——— : Chromosome Aberration Test

Human peripheral blood lymphocytes were stimulated into division in culture then treated with the test articles at a range of concentrations up to 453 μM (206.5μg/ml) based on results from a previous study(study no. 95126, report T3376). Cultures were treated for 4 hours ± rat S9 mix and for 21 hours in the absence of S9. Concurrent vehicle and positive controls were used. The study as reported was adequate.

Cultures treated with clevidipine without degradants showed dose-related and statistically significant increases in the incidence of cells with aberrant metaphases for all treatment conditions. Similar results were obtained for clevidipine + degradants. ———

APPENDIX I Email Correspondence

From: Greg Williams [greg.williams@THEMEDCO.com]
Sent: Tuesday, June 03, 2008 4:08 PM
To: Hausner, Elizabeth A
Cc: Cooper, Monica; Crowley, Alisea; Resnick, Charles A; Lori Lucas; Stuart Abel; Amanda Goodwin
Subject: RE: NDA 22-156, Clevidipine - Response to Questions about the Potential Genotoxicity of Drug Product

Attachments: TMC-R307.pdf; emfalert.txt
Hi Elizabeth,

Thank you for reviewing these reports so promptly. Our responses to your questions are as follows:

First Question:

- **The methods section on page 40 mentions vials 1-5 and notes that individual degradants were placed into individual vials with clevidipine. It is not clear to me what is in each vial, e.g. was it clevidipine +, clevidipine +, and clevidipine + (which is only 3 vials)? This also raises a question as to whether the degradants were tested separately from each other or how they could have been combined to achieve the reported concentrations.**

Response:

- For the Bacterial Mutation Test and Chromosome Aberration Test, each vial (Test Article 2) contained clevidipine with all three degradants.

- In anticipation of these studies being conducted by, eight separate vials containing clevidipine with all three degradants were prepared by for reconstitution in DMSO by These vials were prospectively prepared to facilitate preparation of Test Article 2 solutions without their having to perform multiple weighings. Report number TMC-R307, Attachment 1 (attached, which was prepared by provides the weight of each material which was transferred into each vial. This report also provides the detailed directions which were used for their reconstitution. Following preparation, the 8 vials were shipped (on dry ice) to and stored at 2-6C (in the dark) until use.

- Of the 8 vials prepared by individually reconstituted vials 1-5, and combined these solutions to create the single test solution referenced on page 40 of our submission (page 13 of the Bacterial Mutation Test Report).

- The Chromosome Aberration Test (please see page 112 of our submission, or page 12 of the Chromosome Aberration Test Report) used only the contents of vial number 6, which also contains clevidipine with all three degradants.

Second Question:

- **Please clarify how the test articles were prepared and actually applied to the cell culture plates. I have the same questions regarding methodology for the Chromosome Aberration assay report.**

Response:

- As noted in both study reports, DMSO was the vehicle used for reconstitution of the vial contents (Section 6.1, Vehicle, Bacterial Mutation Test Report page 12, submission page 39 and Chromosome Aberration Test Report page 11, submission page 111).
- For the Bacterial Mutation Test (Bacterial Mutation Test Report pages 16, 17 and 18, submission pages 43, 44 and 45):
    - the test solutions were prepared as noted in Section 7.5 (and as discussed above)
    - using DMSO, the test solutions were serially diluted to the concentrations shown in Text Table 1 (report page 18, following Section 7.9)
    - aliquots of these test solutions (0.1 mL) were then added to approximately 2 mL of agar (Plate Incorporation Method, Section 7.8) or bacteria/S9 mix/buffer (Pre-incubation Method, Section 7.9) to achieve the final concentrations/plate which are also shown in Text Table 1 (report page 18, following Section 7.9).
- For the Chromosome Aberration Test (Chromosome Aberration Test Report pages 14, 15 and 16, submission pages 114, 115 and 116):
    - the test solutions were prepared as noted in Section 7.4 (and as discussed above)
    - using DMSO, the test solutions were serially diluted to the concentrations shown in Text Table 1 (report page 16, following Section 7.7)
    - aliquots of these solutions (10 uL) were then added to each mL of culture (Treatment, Section 7.7) to achieve the final concentrations shown in Text Table 1 (report page 16, following Section 7.7)

We believe these responses completely address your questions and hope that you find this information helpful.

Please let me know if I may provide further assistance with your review.

Thank you,

Greg

From: Hausner, Elizabeth A [mailto:elizabeth.hausner@fda.hhs.gov]
Sent: Tuesday, June 03, 2008 10:47 AM
To: Greg Williams
Cc: Cooper, Monica; Crowley, Alisea; Resnick, Charles A
Subject: RE: NDA 22-156, Clevidipine - Response to Questions about the Potential Genotoxicity of Drug Product

Dear Greg,

I have been reading the reports submitted on 5/30 and need some clarification.

The methods section on page 40 mentions vials 1-5 and notes that individual degradants were placed into individual vials with clevidipine. It is not clear to me what is in each vial, e.g. was it clevidipine + ——, clevidipine + ——, and clevidipine + —— (which is only 3 vials)? This also raises a question as to whether the degradants were tested separately from each other or how they could have been combined to achieve the reported concentrations.

Please clarify how the test articles were prepared and actually applied to the cell culture plates. I have the same questions regarding methodology for the Chromosome Aberration assay report.

Thank you for help with this,

Elizabeth

From: Greg Williams [mailto:greg.williams@THEMEDCO.com]
Sent: Friday, May 30, 2008 4:12 PM
To: Crowley, Alisea
Cc: Hinton, Denise; Karkowsky, Abraham M; Hausner, Elizabeth A; Beasley, Bach Nhi t; Lori Lucas; Stuart Abel
Subject: NDA 22-156, Clevidipine - Response to Questions about the Potential Genotoxicity of Drug Product ——

Dear Alisea,

As previously promised, please find attached our responses to the Pharm. Tox. and CMC questions about the potential genotoxicity of drug product ——. We are also having hard copies of this amendment delivered to the Central Document Room on Monday, 6/2.

We believe that this response addresses the last outstanding questions from the review team and look forward to working with you on labeling.

I will follow-up with you early next week to learn how we can best work together to complete the review process

Thank you and have a great weekend.

Greg

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
6/18/2008 03:12:48 PM
PHARMACOLOGIST
Elizabeth Hausner

Charles Resnick
6/19/2008 03:13:04 PM
PHARMACOLOGIST

Memorandum

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION CENTER FOR DRUG EVALUATION AND RESEARCH DIVISION OF CARDIOVASCULAR AND RENAL PRODUCTS

NDA 22-156

Date: April 3, 2008

From: A. DeFelice, PhD. Supervisory Pharmacologist, DCaRP

To: File: NDA 22-156

Re: Supervisory pharmacologist's overview of Study Report T3367: Mouse micronucleus test of clevidipine (H324/38). Astra safety assessment S-151 85 Sodertalje, Sweden. dated 1October 1996

This memo responds to the review of this study (Study Report T3367 for study no 95115) by Dr. Elizabeth Hausner, the primary pharmacology-toxicology reviewer for NDA22156. In view of unequivocally positive *in vitro* genotoxicity findings (mutagenicity and clastogenicity) in Ames, human lymphocyte, and mouse lymphoma assays, she re-visited this study – the only one which assessed for any *in vivo* genotoxicity – , and recently archived her review (DFS; NDA22156 file; Dr. E.H.: 3/27/08). In it, she questioned the conduct of the assay used, and its suitability for assessing genotoxicity of a formaldehyde pro-drug (the metabolism of this calcium channel blocker affords formaldehyde). Accordingly, she is neither surprised nor re-assured by the negative genotoxicity findings. She had recommended approval from the pharmacology-toxicology perspective pending resolution of the genotoxicity issue.

This memo documents the supervisory pharmacologist's overview of this study, and the empirical basis for concluding the assay was adequately conducted and interpretable, and informs the potential for clastogenic genotoxicity of clevidipine *in vivo*. I find the negative results very re-assuring - especially in the context of other relevant pre-clinical pharmacodynamic and pharmacokinetic studies which reveal the dosages tested as egregiously suprapharmacodynamic and ready access of clevidipine or its metabolites to bone marrow - the tissue monitored in this assay.
[I have already overviewed the empirical evidence that the *in vitro* genotoxic is primarily, if not totally, attributable to the formaldehyde metabolite, and given my empirical basis for concluding - from the much higher endogenous rate of formaldehyde synthesis, and the negative in vivo clastogenicity findings in the subject study - that

clevidipine does not carry a risk of material in vivo genotoxicity (DFS; NDA22156; DeFelice; 3/14/08].

Study Report T3367: Mouse micronucleus test of clevidipine (H324/38). Astra safety assessment S-151 85 Sodertalje, Sweden. dated 1October 1996.

A. Study conduct:
Sponsor asserts that the study was performed per GLP recommendations issued both by the Swedish Medical Products Agency and FDA, and the report bears the signatures of both the VP of safety assessment, and the Quality Assurance manager.
No obvious deviations from the standard conduct of this routine in vivo clastogenicity assay was apparent regarding positive and negative controls, dose selection (close to a lethal dosage), number of mice, sampling of bone marrow, preparation/staining of marrow smears, and microscopy for incidence of micronucleated polychromatic erythrocytes (index of clastogenicity), and polychromatic/normochromatic erythrocyte ratio (marker of erythroblast mitotic index).

B. Dosage tested:
The purity of the batch of Clevidipine used is asserted to be 99.6%., and the compound was said to be stable during the period of dosing.
Up to 82 mg/Kg of clevidipine, infused over approx. 10 min., was administered. This is a very large overdosage vis a vis the 24 and 120 ug/Kg/min. dosage needed to reduce arterial blood pressure by approximately 30% in the conscious hypertensive and normotensive rat, respectively. It also represents an egregiously large multiple of the max. recommended human dose of 4 mg/Kg administered over 24 hours (500 ml of a 0.5 mg/ml intravenous solution given to a patient weighing 60 Kg)

Bone marrow exposure:
As is standard practice, test compound/ metabolite content in the bone marrow was not measured. However, sponsor has performed a relevant radiolabeled study (PK04-095) in the rat. After intravenous infusion of ^{14}-C –clevidipine, labelled such that ^{14}-C would accompany any spontaneous or enzymatically cleaved formaldehyde, the bone marrow proved to be among the most radioactive - and persistently radioactive – tissues. Radioactive content was higher even than in the urine. Accordingly, intravenous clevidipine or its metabolites readily access the rat bone marrow. I see no reason to expect that iv clevidipine would not similarly access the mouse bone marrow.

C. Interpretability of the Assay (criteria for assessing results; effect on mitosis):

Both negative (vehicle) and positive (MMS, a known clastogen) controls were included, and cells harvested both at 24 and, to detect any delayed effects, 48 hours. Both the

negative and positive controls behaved as expected, the MMS provoking excess PCE at the $p<0.01$ significance level. By both Kruskal-Wallis mean rank (Sponsor) and by ANOVA (FDA, at my request), there was no statistically significant excess incidence of micronucleated polychromatic erythrocytes vs. negative control at the critical $p=0.01$ level (standard for this assay). Furthermore, I requested that our statistician also collapse all treated groups and compare to vehicle (since a. all dosages were supra-pharmacodynamic; b. the mean micronucleus incidences were approximately the same in each of the three treated cohorts, and c. dose-relatedness is not necessarily a *sine qua non* for a positive finding). Again, the excess was not statistically significant at the $p<0.01$ level.

Dr. Hausner noted, prudently and plausibly, that any decrease in mitotic index might affect micronucleus production, and confound detection of any excess in the latter. However, the % PCE of all erythrocytes among the 1000 erythrocytes first examined was not depressed vs. control, but rather was comparable across all cohorts, both at 24 and 48 hours. In this assay, the % PCE i.e., PCE/NCE ratio, is a marker of rate of bone marrow erythroblast activity. Accordingly, Sponsor is justified in concluding that clevidipine did not interfere with normal mitotic cell division under the test conditions employed.

D. Suitability and relevance of this assay for formaldehyde pro-drugs.

The primary reviewer was hardly re-assured, and hardly surprised, by the negative findings in this assay, and notes that "cross-linking macromolecules does not necessarily produce micronuclei. Fair enough. However, this is a clastogenicity assay i.e., micronucleus production is a reflection of an egregious cleavage (both strands) of double-stranded DNA. Clevidipine and formaldehyde were certainly capable of such egregious clastogenicity *in vitro* in the human lymphocyte chromosome aberration assay, and I see no reason *a priori* why the mouse erythroblast would not be susceptible to formaldehyde if enough were to "reach" its chromosomes.

Conclusions:

Since formaldehyde and clevidipine +S9 are emphatically capable of egregious mutagenicity and clastogenicity *in vitro* at high enough concentrations in mammalian (including human) cells; and since the formaldehyde pro-drug clevidipine was tested at up to very high multiples of the human and rodent pharmacodynamic dosages in the subject assay; and since the ^{14}C-labeled clevidipine and/or a metabolite readily access the marrow, I interpret the negative findings to the high-reactivity of formaldehyde with proteins - which denies it access to the erythroblast chromosomes - rather than to the assay being insensitive or inappropriate for testing clastogenicity of formaldehyde pro-drugs. Accordingly, I am re-assured by the negative findings in this assay, and would be even more so if the mouse erythroblast were tested *in vitro*, and shown to be vulnerable to clevidipine plus S-9 metabolic enzymes. Certainly the mouse lymphoma cell was susceptible *in vitro* to the genotoxicity of clevidipine + S-9, the latter combination, in

fact, equaling the toxicity of the positive control.

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/s/

Albert Defelice
4/3/2008 04:18:58 PM
PHARMACOLOGIST

Memorandum

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF CARDIOVASCULAR AND RENAL PRODUCTS

NDA 22-156

Date: March 14, 2008

From: A. DeFelice, PhD.
Supervisory Pharmacologist, DCaRP

To: File: NDA 22-156

Subject: NDA22156: formaldehyde metabolite/genotoxicity issue.

This memo responds to the primary pharmacology/toxicology review of NDA 22156 (Dr. E Hausner, DFS: 1/28/2008) which recommends approval, from a pharmacology/toxicology perspective, pending resolution of the perceived genetic toxicology issue. That issue owes, primarily, to formaldehyde being one of the metabolites of clevidipine, and is addressed herein from an exposure perspective:

Ames test: report 961056
This bacterial assay, which monitors reversion to histidine independence in several strains of S. *typhimuriam*, was performed with and without S9. The latter is a hepatic microsomal fraction harvested from Aroclor-induced rats, and added to confer metabolic (mixed function oxidase) capacity. The mutagenicity of clevidipine - manifest in strains TA102, TA98 and, especially, TA100, and only in the presence of S9 metabolizing mix - was completely (strain TA100) or appreciably (strains TA102 and TA 98) obviated in the presence of formaldehyde dehydrogenase (FDH). Accordingly, the mutagenicity of metabolized clevidipine in the several salmonella strains can be importantly attributed to formaldehyde, a recognized metabolite of clevidipine. The primary review notes that mutagenicity of clevidipine +S9 exceeded that of formaldehyde +S9, and that FDH did not completely prevent positive clevidipine findings in all vulnerable strains, which would argue *prima facie* that formaldehyde only partially underlies the positive mutagenicity findings. That may be true. However, it was not established whether the amount of FDH enzyme added was sufficient to maintain clevidipine-afforded formaldehyde titre below a mutagenic threshold. The amount of FDH in the mechanistic assays using such was adequate to obviate the mutagenicity of up to, depending on the S. strain, only 15-30 ug/plate of added formaldehyde, whereas clevidipine was tested up to concentrations of 300 to 900ug/plate depending on the strain. Adding FDH to the assay clearly shifted the clevidipine mutagenicity curves to the right, and, in fact, completely prevented mutagenicity in strain TA100, the strain

most vulnerable to formaldehyde and to clevidipine mutation at the histidine locus (this assay monitors the functional integrity of the latter).

Mouse lymphoma assay: In this assay of mutation at the thymidine kinase locus, clevidipine, in the presence of S9 metabolizing enzyme mix (S9), promoted mean mutant frequency as much as the positive control. Absent S9, activity was still 30% of pos control, but at a concentration that almost stopped cell growth. The role of formaldehyde is unclear as formaldehyde is formed even absent S9, and addition of FDH inhibited cell growth. Nevertheless, FDH completely blocked the mutagenic activity of clevidipine +S9 in the assay. Primary reviewer believes that FDH blunted the sensitivity of the assay by rendering suspension cell growth sub-optimal. However, this reviewer notes that the mutagenic response to the positive control was fully preserved despite the inhibition of suspension cell growth. The primary reviewer suggested that additional concentrations of clevidipine, when tested absent S9, should have been tested in the 37mg/L to 46.2 mg/L concentration to identify concentration – related activity absent S9-enhanced metabolism. However, to this reviewer that would, at most would, only confirm the mutagenicity at essentially one concentration i.e., ca 40 mg/L. At that concentration, mutagenicity was marginal at most; and higher concentrations of clepidine,-S9, virtually stop cell suspension growth. And what could be made of that restricted evidence of marginal mutagenicity, and only at close to an excessively cytotoxic concentration?

Mouse micronucleus assay:
Administered at up to 1/3rd the acute lethal IV dosage, clevidipine was considered to be negative for clastogenicity in the mouse micronucleus test by multiple criteria. Mean incidence in treated cohorts was within historical control, and the highest individual incidence observed in any cohort was comparable, namely 8-10 micronucleated polychromatic erythrocytes/ 2000 examined. Radiolabelled PK studies showed that clevidipine or its metabolites readily accessed the bone marrow.

Studies with human lymphocytes:
Sponsor concluded, and neither primary nor this reviewer challenge, did not promote DNA synthesis in human cultured lymphocytes whether or not mitosis was stimulated by the mitogen phytohemagglutinin (PHA). In report t3367/study report95126, substantial increase in chromosomally-abberent cell incidence, were, again as in other positive genotoxicity assays, observed only in cultures with metabolic activation.
Primary reviewer cites an ostensible increase in mitotic index in a human lymphocyte chromosome aberration assay, and suggests this be put in the mutagenicity labeling. However, such a finding, if real, is not taken as evidence prima facie of genotoxicity. Indeed, the mitogen PHA is routinely included in in vitro chromosomal aberration assays to promote cell cycling and the opportunity to detect any metaphase chromosomal anomalies.

Formaldehyde formation in humans.
Clevidipine is quantitatively converted to its metabolite (H 152/81) with the liberation of formaldehyde *in vitro* in blood with a half-life of 6 minutes. Sponsor reasonably expects that, at the high clinical infusion rate of 8ug/Kg/min (i.e., 17.53nmol/Kg/min), the highest steady-state formaldehyde concentration would be formation rate/clearance rate ratio i.e., 17.53/46.2 = 0.379 nmol/ml. =379nM. Actual human clevidipine Cmax measured by Sponsor was 156 nM, which I

expect to also be the maximum formaldehyde level given the 1:1 generation from clevidipine, and in the absence of any reactivity or metabolism of the aldehyde. This formaldehyde level is ca. 400-600X lower than the endogenous level of 67-100 μM reported (Heck and Casanova, 2004). Accordingly, the extra body burden of formaldehyde afforded by clevidipine metabolism, as calculated, would not add importantly to the body burden of endogenous formaldehyde, as reported – even if the calculated extra body burden were to be appreciably underestimated, and the endogenous rate of formaldehyde production were to be appreciably overestimated.

Dhaareshwar and Stella (2007) have calculated, based on published empirical data, the turnover of endogenously-generated formaldehyde as follows: Using a volume of distribution of 49 L (approx. total body water) and steady-state tissue, cells, and body fluid formaldehyde level of 2.6 mg/L (Heck et al 1985; Casanova-Schmitz et al 1988), Cascieri and Clary (1992) calculated an endogenous total formaldehyde body content of 123 mg (1.75 mg/Kg bw for 70 Kg adult). Since formaldehyde has a 1.5 min half-life, they calculate that 61.25 mg of formaldehyde are turned over every 1.5 min. i.e., 41 mg/min. That is the amount being metabolized or formed to maintain the endogenous steady-state body level of 0.1 mM (2.6 mg/L). *That 41 mg/min of formaldehyde being generated endogenously would be 1108 X greater than the 0.037 mg/min afforded by clevidipine infused at 560 ug/min and assuming that the latter is metabolized, on a 1:1 molar basis, to the aldehyde.*

Formaldehyde exposures: marketed drugs:
The formaldehyde generated in vivo per dose of the prodrugs Cerebyx, Aquavan, Spectracef, Hepsera, and Viread is in the range of 1-103 mg, and includes that afforded by clevidipine, namely ca. 50 mg/day.

Conclusion: In the opinion of this reviewer, the behavior of clevidipine in these mutagenicity and clastogenicity assays, and persuasive evidence that such is attributed primarily, if not exclusively, to formaldehyde at supraphysiological levels during conduct of the in vitro assays, does not project human genotoxic risk at the proposed clinical dosages and duration of exposure. This opinion is intentionally not based on consideration of environmental standards for maximum permissible breathing levels, and a safety multiple relative to such. That is, a 40 minute exposure to 2.5 mg/m^3 – overtly irritating to the eyes, nose, and throat – did not significantly promote mean plasma formaldehyde titre (2.77± 0.28 ug/g vs. baseline of 2.61± 0.14 ug/g: Heck et al, 1985).

It is well recognized that formaldehyde, and therefore clevidipine can be genotoxic *in vitro*, and *a priori* and by extension, potentially *in vivo*. However, the amount of formaldehyde calculated, worst case, to be afforded by clevidipine is much lower than the amounts needed to induce sister chromatid exchange (SCE) in human blood *ex vivo*. According to Schmid and Speit (2007), excess SCE was elicited only at formaldehyde levels > 100uM, and excess micronuclei were not seen even at up to 250 uM. By comet assay, formaldehyde can indeed induce DNA-protein cross-links at ≥ 25uM. However, such cross-links, at least those induced at concentrations up to 100uM, are completely removed, enzymatically or otherwise, before the lymphocytes start to replicate, and therefore is reversible. It is the irreversible toxicologic gene damage which should worry us most from a regulatory risk/benefit perspective.

Heck H,Casanova M. 2000. The implausibility of leukemia induction by formaldehyde: a critical review of the biological evidence on distant-site toxicity.Regul Toxicol Pharmacol. 40(2): 92-106

Schmid O, Speit G 2007. Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. Mutagenesis 22(1): 69-74.

Dhareshwar S , Stella V. 2007. Your pro-drug releases Formaldehyde: should you be concerned? No! J Pharm Sci DOI 10.1002/jps.21319

Heck H, Casanova –Schmitz M et al. 1985. Formaldehyde concentrations in the blood of humans and Fischer 344 rats exposed to CH2O under controlled conditions. Am Ind Hyg Assoc J 46:1-3

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/s/

Albert Defelice
3/14/2008 02:32:29 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22156
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**7/2/07**
PRODUCT:	clevidipine
INTENDED CLINICAL POPULATION:	[redacted]
SPONSOR:	**The Medicines Company**
DOCUMENTS REVIEWED:	**Electronic**
REVIEW DIVISION:	**Division of CardioRenal Drug Products (HFD110)**
PHARM/TOX REVIEWER:	**E.A. Hausner, D.V.M.**
PHARM/TOX SUPERVISOR:	**A.F. DeFelice, Ph.D.**
DIVISION DIRECTOR:	**N. Stockbridge, M.D., Ph.D.**
PROJECT MANAGER:	**Denise Hinton**

Date of review submission to Division File System (DFS):

NDA number: 22156
Review number: 1
Sequence number/date/type of submission: July 2, 2007
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: The Medicines Company
Manufacturer for drug substance:

Reviewer name: Elizabeth Hausner, D.V.M.
Division name: **Division of Cardio-Renal Drug Products**
HFD #:110
Review completion date:

Drug:

Trade name: Cleviprex™
Generic name: clevidipine
Code name: H324/38

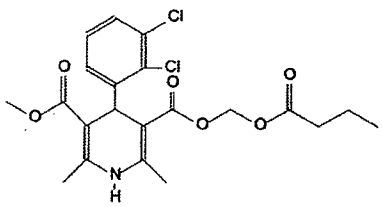
Chemical names:

Butyroxymethyl methyl 4-(2',3'-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate

4-(2',3'-dichlorophenyl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 3-butyryloxymethyl ester 5-methyl ester

CAS registry number: 167221-71-8
Molecular formula/molecular weight:

$C_{21}H_{23}Cl_2NO_6$



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Structure:

Addendum to NDA review

T3367 Mouse Micronucleus Test of Clevidipine

This GLP study was conducted in 1996 and was originally reviewed by Preet Gil-Kumar, the original Pharm-Tox reviewer for this file, in the original submission. It is added to the NDA file for completeness.

Test article: batch 300/94, purity 99.6%
Vehicle: N,N-dimethylacetamide/water (80/20)% w/w
Group size: 7/treatment/concentration/sampling time (n=63 total in main study)

The effect of clevidipine on the incidence of micronucleated polychromatic erythrocytes was studied in male mice. Doses of 8.2, 41 and 82 mg/kg were given intravenously. Vehicle was used as the negative control and methyl methanesulfonate (MMS) as positive control(given by oral gavage). Mice were euthanized either 24 or 48 hours after a single dose. Bone marrow smears were prepared and stained. The ratio of polychromatic to all erythrocytes was assessed by examination of the first 1000 erythrocytes observed. A total of 2000 polychromatic erythrocytes from each animal were examined for the presence of micronuclei.

The sponsor did not provide criteria by which they were to judge:

- Validity of the study
- A positive test result
- A negative test result
- An equivocal test result

Could a pertinent comparator compound, e.g. another drug that generates formaldehyde, or formaldehyde itself, have been used?

In the preliminary toxicity testing preceeding the micronucleus test:
2 of 10 animals dosed with 300 μmol/kg died
2 of 3 animals given 240 μmol/kg died

10/10 180μmol/kg all survived but showed decreased motor activity and decreased respiratory frequency. The vehicle animals reportedly showed the same signs.

While there was no apparent effect at 24 hours, there was an increase in mean MPCE at 48 hours in all drug-treated groups. The vehicle control showed mean MPCE of 3.3 while the drug treated groups showed mean MPCE of 5.1-5.3.

RESULTS FROM THE ANALYSIS OF THE 48-HOUR SAMPLING

Test compound	Sampling time h	Dose µmol/kg	Animal No. and sex	MPCE(T) No.	MPCE(T)+ PCE(T) No.	POLY %	MNCE No.	NORMO No.
Vehicle	48	0	5658/95 M	2	2000	31.8	0	682
			5659/95 M	0	2000	77.3	0	227
			5660/95 M	3	2000	35.2	0	648
			5661/95 M	4	2000	29.4	0	706
			5662/95 M	2	2000	44.5	0	555
			5663/95 M	9	2000	33.6	0	664
			5664/95 M	3	2000	29.4	0	706
H324/38	48	18	5665/95 M	6	2000	46.4	1	536
			5666/95 M	3	2000	48.8	0	512
			5667/95 M	8	2000	40.5	2	595
			5668/95 M	7	2000	36.2	0	638
			5669/95 M	5	2000	28.1	0	719
			5670/95 M	3	2000	29.3	0	707
			5671/95 M	4	2000	55.6	1	444
H324/38	48	90	5672/95 M	6	2000	44.2	1	558
			5673/95 M	9	2000	45.2	0	548
			5674/95 M	4	2000	21.8	2	782
			5675/95 M	2	2000	37.8	0	622
			5676/95 M	10	2000	40.7	0	593
			5677/95 M	3	2000	44.6	0	554
			5678/95 M	3	2000	57.1	0	429
H324/38	48	180	5679/95 M	8	2000	46.7	0	533
			5680/95 M	5	2000	33.7	2	663
			5681/95 M	2	2000	55.3	0	447
			5682/95 M	8	2000	44.9	1	551
			5683/95 M	6	2000	35.0	0	650
			5684/95 M	2	2000	48.1	0	519
			5685/95 M	6	2000	43.2	0	568

MNCE = Micronucleated normochromatic erythrocytes observed
MPCE(T) = Total number of micronucleated polychromatic erythrocytes within all polychromatic erythrocytes examined
NORMO = Normochromatic erythrocytes of all erythrocytes among the 1000 erythrocytes first examined
PCE(T) = Total number of polychromatic erythrocytes without micronuclei within all polychromatic erythrocytes examined
POLY = Polychromatic erythrocytes of all erythrocytes among the 1000 erythrocytes first examined

The range of historical controls was 0-8. So while 3 animals exceeded the upper limit of the historical controls (including 1 in the vehicle control group) and 3 animals met the upper limit, there is not a robust, dose-related effect.

The vehicle used is different from that used in the tox studies. The sponsor's lack of criteria for assessing the results is suboptimal at best. With hindsight, it would have been advisable to examine a longer term standard tox study for micronuclei to confirm or contradict the results of this assay. This also needs to be viewed in light of study T3363 in which there were changes in mitotic indices which the sponsor interpreted as mitotic arrest. There was no information in this 22 page report to suggest that the sponsor had given this previous observation any special consideration. If the rate was slowed down due to mitotic arrest, would that affect micronucleus production? However, I don't see that this changes the fact of formaldehyde produced as a metabolite of clevidipine. Cross-linking macromolecules does not necessarily produce micronuclei so the negative result in this assay is not particularly surprising.

The certificate of analysis also does not list any impurities, either those cited by the chemists as impurities of process or those reported as degradants. Also, as noted in the draft guidance for genotoxic impurities, the genotoxicity of even those chemicals used as positive controls can be missed if not tested in appropriate amounts (that is "spiked"). Therefore, while this assay may be viewed as negative, it does not apparently address the 5 chemical species (2 process impurities and 3 degradants) yet to be characterized.

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/s/

Elizabeth Hausner
3/27/2008 07:42:27 AM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
3/27/2008 09:50:11 AM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22156
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PRODUCT:	clevidipine
INTENDED CLINICAL POPULATION:	
SPONSOR:	**The Medicines Company**
DOCUMENTS REVIEWED:	**Electronic**
REVIEW DIVISION:	**Division of CardioRenal Drug Products (HFD110)**
PHARM/TOX REVIEWER:	**E.A. Hausner, D.V.M.**
PHARM/TOX SUPERVISOR:	**A.F. DeFelice, Ph.D.**
DIVISION DIRECTOR:	**N. Stockbridge, M.D., Ph.D.**
PROJECT MANAGER:	**Denise Hinton**

Date of review submission to Division File System (DFS):

TABLE OF CONTENTS

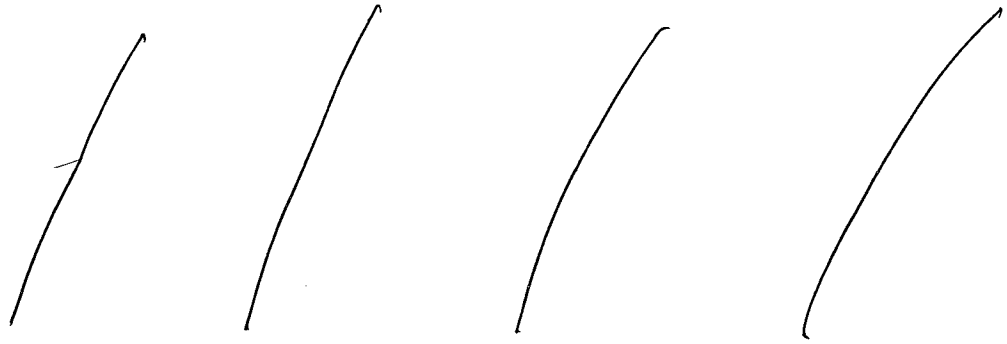
EXECUTIVE SUMMARY

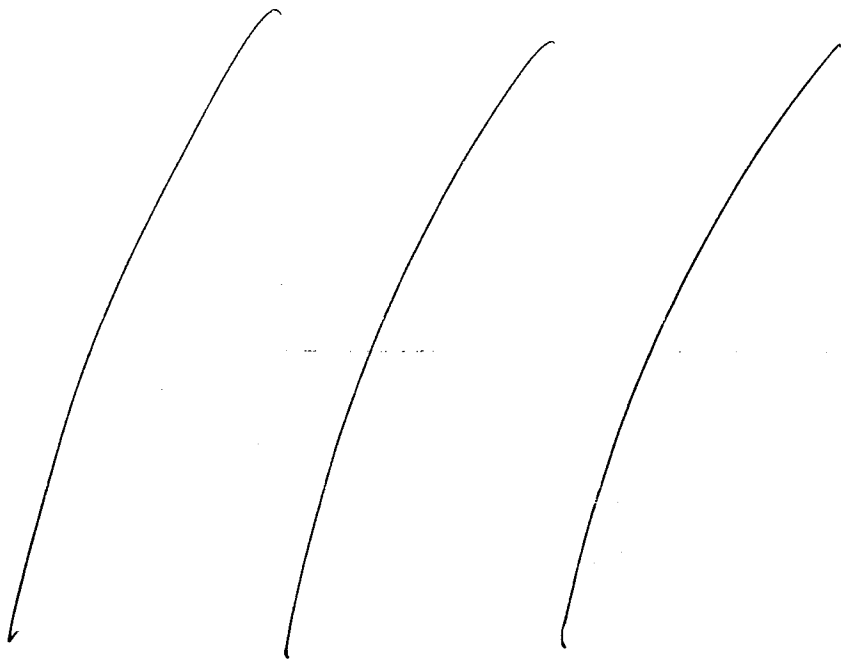
I. Recommendations

A. Recommendation on approvability: *Approvable with resolution of the genotoxicity issue.*

B. Recommendation for nonclinical studies: *An in vitro receptor binding study should be provided. If further studies are required to address the level of concern for the formation of formaldehyde, clinical work would seem more direct.*

C. Recommendations on labeling:





II. Summary of nonclinical findings

A. Brief Overview of Nonclinical Findings

Safety Pharmacology
The safety pharmacology was incomplete. Neither pulmonary nor overt behavioral effects have been studied. The assessment of cardiovascular effects was done in an atypical manner but showed no discernible QTc lengthening or other adverse effects.

A dose-related inhibition of gastric emptying and propulsive small intestinal movements was seen. This is most likely consistent with other calcium channel blockers and the propensity to cause constipation.

Pharmacokinetics/Toxicokinetics
Clevidipine is hightly protein bound (>99.5%) in all species examined. The sponsor states that the drug is not widely distributed (0.45 L/kg at steady state in the rat, 0.17L/kg in the rabbit and 2 L/kg in the dog). However, the radiolabel distribution study showed rapid and widespread distribution of drug-associated radioactivity. The radio-labeled portion of the molecule ($^{14}CH_2O$) persisted in the various tissues sampled until the last point of determination (28 days). The sponsor's statement that radioactivity is completely eliminated within 8 days is not consistent with the data.

H152/81, the main metabolite from all the pre-clinical species, is produced in a 1:1 ratio with the parent drug. The conversion occurs by ester hydrolysis. Formaldehyde is also generated as a metabolite. I would expect formaldehyde to be produced in the same 1:1 ratio. To date, the sponsor has provided theoretical "worst case" calculations of the amount of formaldehyde produced and the effect on the endogenous levels.

General Toxicology

Assessing the general toxicology was confounded by the use of Intralipid as the vehicle. The vehicle caused clinical chemistry and histologic effects consistent with its lipid dense nature. The vehicle also appeared to exacerbate some effects of clevidipine. Another confounding item is the poor quality of conduct of some of the toxicology studies. Animal husbandry techniques can be surmised to have been sub-optimal to atrocious as evidenced by studies with almost universal sepsis, catheter closures, keratitis, poor overall survival and precipitation of drug material in the catheters. The main non-clinical issues are reproductive effects (pseudopregnancy, irregular estrous cycles) and genotoxicity.

Genetic Toxicology

Formaldehyde is produced as one of the metabolites. ACGIH lists formaldehyde as a suspected human carcinogen (A2). EPA calls formaldehyde a probable human carcinogen(B1). The GeneTox database lists positive genotoxicity findings for formaldehyde for sister chromatid exchange done in human cells including lymphocytes and for non-human cells. Other assays were listed as having been done pre-1980. There were a plethora of positive genotoxic assays in this NDA. Formaldehyde was used as a positive control in some of the assays and formaldehyde dehydrogenase was used to determine if the positive results were attributable to the formaldehyde. In some cases, clevidipine produced greater positive responses than formaldehyde alone. The use of FDH did completely resolve the positive assay results. Therefore, either there are other genotoxins in the drug substance, or, clevidipine itself has some genotoxic potential.

A time-weighted average (TWA) is an industrial hygiene/occupational safety recommendation for exposure when a person is expected to work an 8 hour day, 5 days a week and is exposed to some substance of toxic potential. For formaldehyde, NIOSH recommends a TWA of 0.016ppm. For a 15 minute interval they recommend an upper limit of 0.1 ppm. These were the only published guidelines I found for assessing a worst case formaldehyde production with clevidipine use. Making a worst case assumption that 1 molecule of clevidipine gives 1 molecule of formaldehyde the exposure is summarized in the reviewer's table below:

Proposed dose	Dose in mg/kg for a 60 kg patient	Dose relative to the TWA
2 mg/hour	0.033	1X in 1 hour
32 mg/hr	0.53	16X in 1 hour

Conversion factor: 1ppm=1mg/kg

Reproductive Toxicology

The effects seen were typical of those seen with other calcium channel blockers: dystocia, delayed parturition, impaired male fertility. Something that appears to be unique to clevidipine is pseudopregnancy in rats and unusual or atypical estrous cycle length. This is summarized in the table below. There were 19 animals total per group.

Number of Animals Showing Oestrous Cycle Irregularities During Treatment

Dose Group	Number Pseudopregnant	Number with Cycles of Unusual Duration
1	0	0
2	3	4
3	2	2
4	3	2

B. Pharmacologic Activity

Clevidipine is a dihydropyridine calcium channel blocker prepared as a racemic mixture of the R- and S- enantiomers. The enantiomers are apparently of equivalent pharmacological and pharmacokinetic properties. The major metabolite, M1(H152/81) had no discernible effect on blood pressure when given in a molar dose 70X higher than that required for clevidipine to lower arterial blood pressure by 30%. The receptor binding profile of clevidipine and the M1 metabolite is unknown as the sponsor did not conduct this particular study.

C. Non-Clinical Issues Related to Clinical Use

The generation of formaldehyde as a metabolite is pertinent to clinical use. The question to be resolved is whether the levels of formaldehyde produced in the infusion are of clinical concern. The reproductive toxicity issues can be addressed in labeling.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22156
Review number: 1
Sequence number/date/type of submission: July 2, 2007
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: The Medicines Company
Manufacturer for drug substance:

Reviewer name: Elizabeth Hausner, D.V.M.
Division name: **Division of Cardio-Renal Drug Products**
HFD #:110
Review completion date:

Drug:

Trade name: Cleviprex™
Generic name: clevidipine
Code name: H324/38

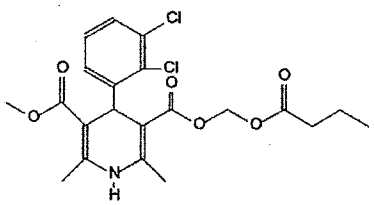
Chemical names:

Butyroxymethyl methyl 4-(2',3'-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate

4-(2',3'-dichlorophenyl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 3-butyryloxymethyl ester 5-methyl ester

CAS registry number: 167221-71-8
Molecular formula/molecular weight:

$C_{21}H_{23}Cl_2NO_6$



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Structure:

Relevant INDs/NDAs/DMFs: IND65,114

Drug class: dihydropiridine calcium channel blocker

Intended clinical population: patients [redacted]
for whom oral therapy is not possible or [redacted]

Clinical formulation: soybean oil, glycerin, purified egg yolk phospholipids, sodium hydroxide

Route of administration: intravenous. Initiated at 2 mg/hour and titrated to effect. Maximum recommended rate of infusion is 32mg/hour with most patients reaching desired response at 16 mg/hour.

Studies reviewed within this submission:

Studies not reviewed within this submission:

- **Studies previously reviewed have not been re-examined**
- [redacted]

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary Clevidipine is a dihydropyridine calcium channel blocker. It has been prepared as a racemic mixture of the R- and S- enantiomers. The sponsor contends that the enantiomers are of equal pharmacokinetic and pharmacodynamic properties. The racemic mixture decreased the L-type calcium current in myocytes. The major metabolite, M1 (H152/81) is reported to have no effect on blood pressure when given in a molar dose 70x higher than that required for clevidipine to lower arterial blood pressure by 30%. Potency is greatest in anesthetized SHR rats, followed by conscious SHR and lowest in normotensive rats. The receptor binding profile of clevidipine and the major metabolite is unknown. The sponsor was requested to supply this information. The sponsor's reply to this request may be seen in Appendix I.

☼ For the studies reported below, clevidipine was formulated in 20%Intralipid® unless otherwise noted.

Slightly expanded summary of pharmacology

The sponsor used a number of in vitro studies to demonstrate that clevidipine is a calcium channel blocker. Cultured cortical neurons were used to demonstrate clevidipine's effect on the potassium-induced calcium influx in primary cortical neuronal cells from neonatal rats. Felodipine was used as a comparator. Isolated rabbit myocytes were used in the whole cell voltage clamp studies demonstrating interaction with the L-type calcium channel. Nicardipine was used as a reference compound. The isolated rat portal vein and paced rat papillary muscle were used to demonstrate that clevidipine behaved similarly to other calcium channel antagonists as far as effect on vascular smooth muscle, inhibiting both spontaneous and induced contraction to some extent. The smooth muscle agonists used were noradrenaline, acetylcholine, PGF2α, serotonin, and angiotensin. Felodipine was used as a comparator for the papillary muscle effects.
Rat papillary muscle and portal vein were also used in an organ bath preparation for comparison of myocardial versus vascular selectivity. Felodipine, clevidipine and vehicle were compared with regards to effect on spontaneous myogenic activity of the portal vein and contractility of the papillary muscle. The sponsor summarized the inhibitory effects on vascular smooth muscle and myocardium in the following table. Not all terms/letters were defined:

Table 2: [Report 2220-0853-01 Section 5.2.2, Table II] Inhibitory potencies (pIC_{50}) of clevidipine and felodipine in the portal vein (PV) and papillary muscle (PM)

	A	B	C	D	E
Group	PV pIC_{50}	PM pIC_{50}	A-B	10^C "selectivity"	Confidence interval for C
Clevidipine	6.37	4.69	1.68	48	1.49-1.87
Felodipine	7.61	5.51	2.10	126	1.81-2.39

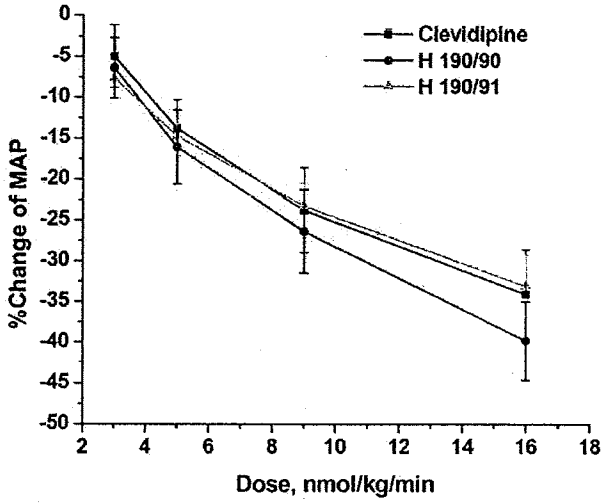
PV = portal vein; PM = papillary muscle

The sponsor presents this as supporting vascular selectivity.

The sponsor then moves on to whole animal studies to demonstrate systemic effects. The sponsor made several necessary comparisons. First clevidipine in a lipid vehicle was compared to clevidipine in an aqueous vehicle. The M1 metabolite was also tested, using an aqueous vehicle. The R- and S- enantiomers were also examined. The effect of the drug in anesthetized normotensive vs anesthetized spontaneously hypertensive rats was compared as well as conscious vs anesthetized SHR.

In anesthetized normotensive and SHR rats, the lipid versus aqueous vehicle did not have a perceptible effect on blood pressure under the conditions of the study. The M1 metabolite did not show an appreciable effect at approximately 70x the concentration of parent drug needed to produce a 30% decrease in blood pressure. The R- and S- enantiomers were compared for effect in anesthetized SHR. There were no appreciable differences between the enantiomers alone or combined as clevidipine. The sponsor's graphical presentation is shown below. Differences between the curves may be due to the variability inherent in the model.

Figure 9: [Report 696 Section 5.2, Figure 2] Effects of increasing infusion rates of clevidipine and its enantiomers (H 190/90 and H 190/91) on mean (SD) arterial pressure (MAP) in spontaneously hypertensive rats (n=6)



In anesthetized SHR, 2 nmol/kg/min caused a 20% decrease in mean arterial pressure and heart rate was unchanged. In conscious SHR, 23 nmol/kg/min was needed to cause a 20% decrease in mean arterial pressure and reflex tachycardia was reported. The difference in drug potency between conscious and anesthetized SHR was attributed to activation of autonomic reflexes in the conscious rats. The potency was also greater in SHR versus normotensive rats. Clevidipine produced a mean 33 % decrease in blood pressure in SHR and a mean decrease of 28% in normotensive rats but at concentrations of 58±8.7 nmol/kg and 316±57 nmol/kg respectively.

Clevidipine was also tested in conscious, normotensive dogs. The dose range of 20-30 nmol/kg/min caused a blood pressure decrease of 20% with a corresponding 80% increase in heart rate.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: dihydropyridine calcium channel antagonist

Drug activity related to proposed indication: vasodilation causing a decrease in arterial blood pressure

Report 2220-870-00/Study 50118 Effect of short-acting dihydropyridine H324/38 on potassium-induced calcium influx in primary cortical neuronal cell cultures from neonatal rats Calcium influx in cultured cortical neuronal cells was induced by exposing the cells to 55 mM potassium. The intracellular calcium concentration was measured directly by a fluoro-3 technique (Fmin determined by incubation with $CuSO_4$ followed by observed relative fluorescence. Fmax determined by incubation with ionophore A-23187, which allowed sufficient influx of calcium to obtain saturation level of binding of the intracellular Fluo-3 ligand. Intracellular calcium calculated by the following equation: $[Ca2+]_i = K_d(F-F_{min})/(F_{max}-F))$.

Intracellular calcium-concentration was decreased with both clevidipine and felodipine. Maximum inhibition reached a mean of 22±11.4 % of control (3 replicates) at a concentration of 40 μM clevidipine. Total inhibition was not reached with either drug based upon the data presented.

	Max inhibition % of control	IC50 (μM) clevidipine	IC50 (μM) felodipine
mean±SD	22.7±11.4	5.77±2.39	1.18±1.18

Report 2220-0853-01/Study 40270: Effects on the paced rat papillary muscle and on spontaneously myogenic activity and induced vasomotor responses in isolated rat portal vein Drug concentrations studied were from 1nM to 10 μM and inhibitory effects of clevidipine on the basal, spontaneous vascular smooth muscle activity of the rat portal vein were compared to contractile responses to vasomotor stimulators (noradrenalin, acetylcholine, angiotensin I, serotonin and PGF2α). The inhibitory effects of clevidipine on spontaneous myogenic activity of the rat portal vein and the contractions of paced rat papillary muscle were compared to determine a vascular vs myocardial selectivity ratio. Clevidipine was dissolved in aqueous 80% PEG400. The portal vein was an isolated organ preparation attached to a force-displacement transducer. The papillary muscle (left ventricular) was also tested in an organ bath, pre-stretched to a passive force of 8mN, under continuous stimulation at 3 Hz. Nerve stimulation and pharmacologic agonists were applied to produce ~70-80% of the maximum response to each means of stimulation. Contractile activity was expressed as a percent of control.

Increasing concentrations of clevidipine inhibited spontaneous and induced responses of the portal vein (up to 100%). Felodipine was used as a comparator compound for the myocardial effects. The graphs of results are very similar for the two compounds. The sponsor felt that the differences in IC50 values for the different tissues were sufficient to describe a selectivity factor. The results are summarized in the table below.

Table II - Inhibitory potencies (pIC_{50}) of H 324/38 and felodipine in the portal vein (PV) and papillary muscle (PM)

Group	A	B	C	D	E
	PV IC_{50}	PM IC_{50}	A-B	10^C "selectivity"	Confidence interval for C
H 324/38	6.37	4.69	1.68	48	1.49-1.87
Felodipine	7.61	5.51	2.10	126	1.81-2.39

Individual values are given in Appendix 3.

Report 2220-0846-00/Study 40252 Antihypertensive potency and effect duration of clevidipine and reference compounds in anesthetized normotensive and spontaneously hypertensive rats.

The effective duration of clevidpine was determined in anesthetized normotensive or SHR rats and compared to nitroglycerin, nitroprusside, felodipine, nicardipine and isradipine. Blood pressure effects of the M1 metabolite were also determined. The drugs were infused at increasing rates in 5 steps over a period of 15 minutes to obtain a blood pressure decrease of 30%. The five infusion rates were (µl/min): 10, 18, 32, 56 and 100. A sixth infusion rate of 300 µl/min was used if the desired blood pressure effect was not achieved. Duration of effect was estimated after termination of drug infusion by the time required for mean arterial blood pressure to increase from a reduction of 30-10% below basal blood pressure. Antihypertensive potency was defined here as the accumulated molar dose of drug required to lower mean arterial blood pressure 30 percent. Clevidipine was dissolved in ——— and then dispersed in 20% Intralipid®.

Results

Table II - Basal blood pressure and percentage change in blood pressure during drug infusion in the various groups

	Basal blood pressure mm Hg	% change from basal value
H 324/38 in Intralipid® - SHR	165±18	-34±3
H 324/38 in Intralipid® - SPDR	115±14*	-28±3 *
H 324/38 in - SHR	163± 8	-32±1
Nitroprusside	173±15	-35±4
Nitroglycerin	185±10	-30±2
Felodipine	167±13	-33±2
Isradipine	171± 8	-32±1
Nicardipine	166± 8	-33±2
H 152/81	183± 7	-2±5
Intralipid®	163±13	0±6
	173±18	-2±4

Individual values are given in Appendix 2.
Basal blood pressure; mean arterial blood pressure as averaged during the 10 min preceding start of drug infusion. The changes from basal value were those obtained at the end of drug infusion or after infusion of vehicle (Intralipid® and —— . * p<0.05 compared to H 324/38 in Intralipid® given to SHR.

Table III: Recovery time and potency

	Recovery time, min	Potency nmol/kg
H 324/38 in Intralipid® - SHR	2.4 ± 0.6	58 ± 9
H 324/38 in Intralipid® - SPDR	3.0 ± 0.6	316 ± 57*
H 324/38 in - SHR	2.4 ± 0.8	59 ± 8
Nitroprusside	0.6 ± 0.2*	184 ± 32*
Nitroglycerin	3.9 ± 3.0	2315 ±2106*
Felodipine	58.7 ± 26*	26 ± 11*
Isradipine	43.4 ± 26*	17 ± 2*
Nicardipine	18.7 ± 8.3*	55 ± 18

* p<0.05 compared to H 324/38 in Intralipid® given to SHR.
Individual values are given in Appendix 3.

Clevidipine produced a mean 33 % decrease in blood pressure in SHR (58±8.7 nmol/kg) and a mean decrease of 28% in normotensive rats(316±57 nmol/kg). The potency in SHR is about half that of felodipine and isradipine and approximately equivalent to nicardipine. The recovery time was very rapid, indicating a short duration of action.

Clevidipine is hydrolyzed to the acid on an equimolar basis.
The main metabolite H152/81 had no apparent effect on blood pressure when given in a molar dose 70 times higher than that of clevidipine required to lower blood pressure 30%.

Report 696/Study 40252 Anti-hypertensive effects of the enantiomers in anesthetized spontaneously hypertensive rats.

The potency and effect of duration of clevidipine and the enantiomers H190/90 and H190/91 were compared in anesthetized SHR. Each rat received all 3 drugs consecutively, infused at increasing rates over a period of 15 minutes with one hour between the different drug infusions. Anti-hypertensive potency was defined as the dose required to lower blood pressure 20% (ED_{20}) expressed in nmol/kg/min. Effect duration was estimated after termination of drug infusion by the time required for mean arterial blood pressure to increase from the achieved reduction below basal blood pressure.

	clevidipine	H190/90	H190/91
ED_{20} (nmol/kg/min)	6.9±1.7	7.3±1.8	7.9±1.9
Recovery time(min)	2.0±0.4	2.3±0.4	2.3±0.3

The sponsor concludes no differences between the 3 drugs with respect to potency or duration of effect. I agree with the duration of effect being the same, but in another study the sponsor has used similar differences in effective concentrations to claim tissue specificity. For consistency, it could be said that there is a slight decrease in efficacy of the individual enantiomers vs the racemic mix under the conditions of the study. Otherwise, the differences are most likely within the variability of the model.

Report 2220-0843-00/Study 50199 Effect on blood pressure and heart rate in conscious and anesthetized rats.

Increasing doses of clevidipine were infused continuously for 30 minutes at 30 minute intervals during continuous intraarterial measurements of MAP and heart rate. Antihypertensive potency was defined by the interpolated dose decreasing MAP by 20%.

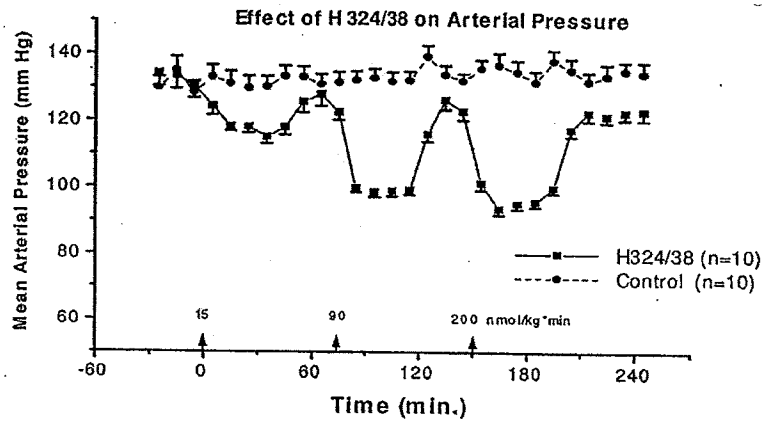
Summary of results

parameter	Anesthetized SHR	Conscious SHR
Dose causing MAP ↓20%	2 nmol/kg/min	23 nmol/kg/min
Heart rate	unchanged	tachycardia

The drug was approximately 10X less potent in the conscious SHR, possibly due to the engagement of autonomic nervous system reflexes.

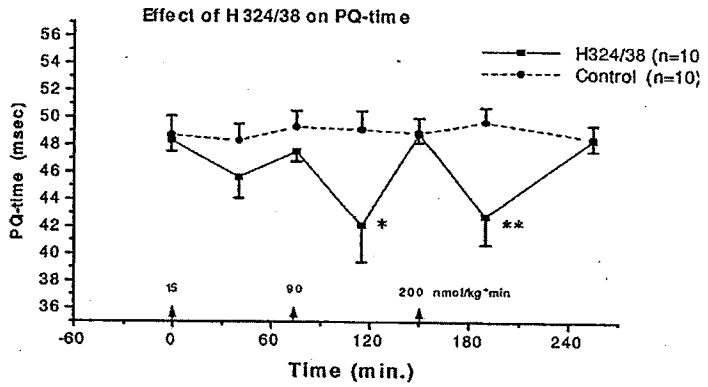
Report 2220-857-00/Study 50199Effect on arterial blood pressure, heart rate, ECG, body temperature, acid-base balance and plasma electrolytes of intravenous infusion of clevidipine in conscious rats

Clevidipine was infused into conscious male SHR at 3 dose rates for 45 minutes at each dose with a 30 minute recovery period between doses. The initial dose was 15 nmol/kg/min followed by 90 and 200 nmol/kg/min. Arterial blood pressure and heart rate were measured continuously via indwelling catheters. An average ECG was obtained before and during the infusion of each dose. Blood samples were also taken for measurement of the acid-base balance, blood gases and plasma electrolytes at each dose level. Body temperature was measured before and during infusion.
The concentrations caused dose-related decreases in MAP. All changes were significant at $p<0.01$.



Corresponding increases in heart rate were seen with each infusion.

As may be expected from an increased heart rate, PQ-time was decreased.



Effect of infusion of H 324/38 or vehicle on PQ-duration from the averaged ECG-recordings. The corresponding numerical data are presented in the appendix to fig 5. (* indicate $p<0.05$, ** indicate $p<0.01$).

There was no consistent or apparent effect on QRS interval.

There are no significant changes in the measured parameters.

Summary of acid-base parameters

parameter	control	End of infusion
pH	7.48±0.01	7.42±0.01
pCO2	3.97±0.35	4.36±0.32
pO2	12.69±0.49	13.10±0.50
BE	-0.17±1.89	-2.13±1.46
Na	144.2±1.14	142.1±1.60
K	2.79±0.28	2.85±0.35

Consistent with the other pharmacology studies, the ECG findings are compatible with a calcium channel blocker.

Report 220-0852-00/Study 40267/40269 Dose finding study for determination of effects on blood pressure and heart rate in the conscious dog

Three dogs were given IV infusions of clevidipine at increasing doses (10, 20, 30, 50, 100 and 600 nmol/kg/min). The infusion duration at each dose level was 45 minutes with a recovery time of 35 minutes between doses. Three infusions were performed per experiment. Results were expressed as a percentage of the pre-infusion value.

The highest dose of 600 nmol/kg/min caused sedation with arterial blood pressure decreased by up to 45%. Blood pressure did not return to baseline values within 30 minutes. The dose range of 20-30 nmol/kg/min caused a blood pressure decrease of 20%. At this level of decreased BP, heart rate was increased by 80%. Infused clevidipine decreased blood pressure in conscious, normotensive dogs.

Report 2220-0869-00/Study50203 Effects on arterial blood pressure, heart rate and ECG of sustained IV infusion in conscious rats

Clevidipine was infused for 11.5 hours at a dose of 65 nmol/kg/min to conscious male SHR. This is about 3 times the predicted therapeutic dose in conscious rats. Arterial

blood pressure and heart rate were measured continuously with indwelling catheters. Averaged ECG data was obtained 3 times during the 24 hour experiment.

Clevidipine produced a 30-35% decrease in arterial pressure with a reflex increase in heart rate. After the cessation of the infusion, blood pressure returned to 90% of baseline within 24.2±7.5 min. Mean arterial blood pressure had not fully returned to baseline within one hour of the end of the infusion. Within the time of observation rebound phenomena were not reported. PQ interval was decreased on the ECG, possibly related to increased heart rate. No effects on QRS and no arrythmias were reported.

Report 2220-854-00/Study 10171 Effect of clevidipine on the L-type calcium channel current in rabbit ventricular myocytes: comparison with nicardipine.

The studies were performed in isolated rabbit myocytes using whole cell voltage clamp. Both drugs were dissolved in ethanol. Calcium current amplitudes were normalized to the calcium current amplitude at 25 minutes after rupture. Holding potential was maintained at -50mV to keep the fast sodium current inactivated. To activate the L-type calcium channel, test pulses with a duration of 250 ms were given to 0 mV. Pulses were given continuously at a frequency of 0.1Hz. At 35, 45 and 55 minutes after rupture, pulse trains of 50 pulses at 1 Hz were transiently given to see if an increased frequency would result in an increase in developed block. At the end of the pulse train increased concentration of drug was administered.

Calcium current showed a concentration related decrease (block) during the low frequency pulses for clevidipine (H324/38) and the pulse train for nicardipine.

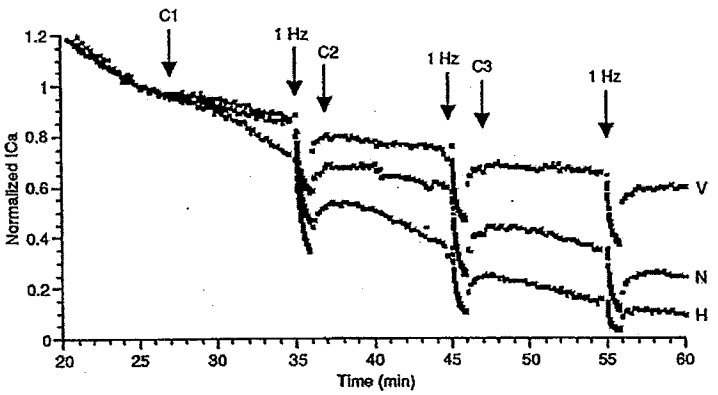


FIGURE 2 Normalized amplitudes of peak currents in control cells given only vehicle (V) and in cells given nicardipine (N) or H 324/38 (H). C1 to C3 mark time for addition of increased concentration of drug. Concentrations for nicardipine were 10, 30 and 100 nM and for H 324/38 the concentrations were 30, 100 and 300 nM, for C1, C2 and C3, respectively. Pulse trains at 1 Hz were performed where marked. Values are mean values of five experiments of amplitudes normalized to the amplitude at 25 min after rupture.

The sponsor noted that the block by nicardipine seemed to develop mainly during the pulse trains and to a lesser extent during the low frequency stimuli. Clevidipine on the other hand, seemed to block the current during both the high and low frequencies. The sponsor felt that this might be indicative of a difference in the development of the calcium channel blockade.

Ratios between the current with drug present and control current were calculated and the data was fitted to the general equation of receptor occupancy.

parameter	nicardipine	Clevidipine
Hill coefficients for best fit	1.2	0.9
Km	39nM	38nM

The Km here is somewhat different than that reported for the papillary muscle (pIC50=5). The sponsor feels that characteristic of the dihydropyridine calcium channel blockers is binding to the inactivated channel state. Therefore, it is postulated that the block will show strong voltage dependence and the results will depend upon the holding potential used.

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2.6.2.3 Secondary pharmacodynamics

Report 2220-0840-01/ Study 50194 Pharmacokinetics and effects on hemodynamics and autonomic nervous control in the anesthetized dog

Anesthetized Beagles of both sexes were given either IV clevidipine or sodium nitroprusside according to the following summary table:

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Drug	Dose No.	Dose nmol/kg/min	Infusion time according to schedule min	Concentration of infusate µmol/ml	Infused volume infusate ml/kg/h	Infused volume of vehicle ml/kg/h
Control	0	0	0 - 45	0	0	0
H 324/38	I	6	45 - 90	1	0.36	-
"	II	18	90 -135	3	0.36	-
"	III	54	135 -180	3	1.08	-
Control	0	0	0 - 45	0	0	0
Nitroprusside	I	7	45 - 90	1.74	0.24	0.36
"	II	21	90 -135	3.48	0.36	0.36
"	III	63	135 -180	3.48	1.08	1.08
Control	0	0	0 - 45	0	-	0
Vehicle	I	0	45 - 90	0	-	0.36
"	II	0	90 - 135	0	-	0.36
"	III	0	135 - 180	0	-	1.08

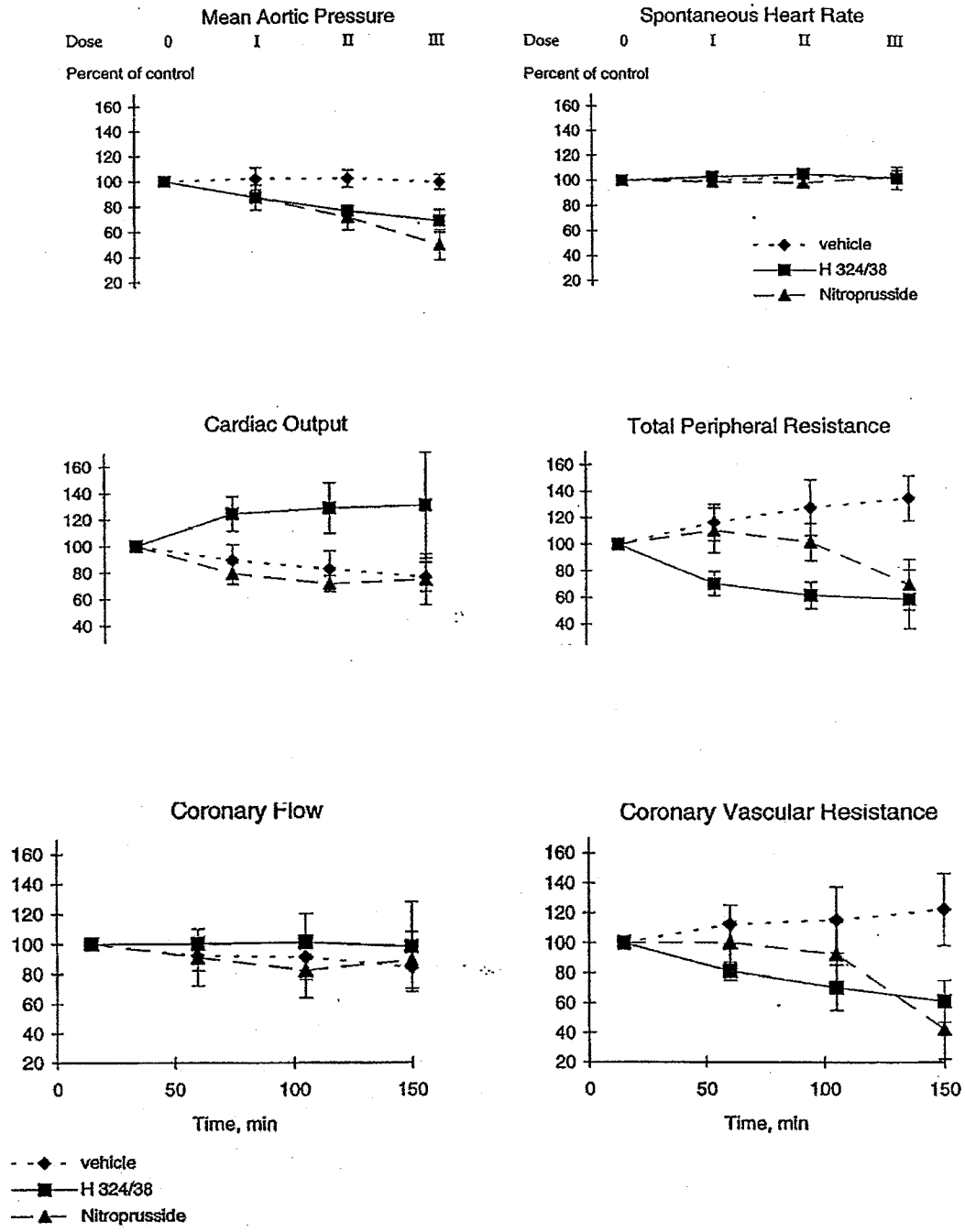
Under anesthesia, the left and right stellate ganglia were exposed and all branches cut except for the cardiac sympathetic nerve. Bilateral vagotomy was performed with bipolar electrodes placed on the distal nerve ends for later stimulation of vagal nerves to the heart.

Four different procedures, each lasting 1 minute were performed:

1. cardiac pacing at 167 bpm
2. bilateral stimulation of cut vagal nerves to obtain a heart rate decrease of ~35 bpm in the control situation
3. bilateral stimulation of the cut sympathetic nerves to obtain a heart rate increase of ~80bpm in the control situation
4. bilateral occlusion of the carotid arteries

This sequence of 4 procedures was repeated in each 45 minute infusion period (4X per animal). 1st sequence= individual control. The sequence was repeated either in the presence of vehicle or increasing dose of either drug. Blood samples were collected at 30 and 36 minutes after the start of infusion of each dose. After dose III, blood was collected at 1,2,4, 6 and 15 minutes after the end of infusion. Blood samples were also collected for analysis of triglycerides and non-esterified fatty acids 30 minutes after the start of each infusion and 60 minutes after the end of the Dose III infusion. All results were expressed as a percentage of the control values.

Aortic pressure showed a dose-related decrease with both drugs but cardiac output increased only with clevidipine. Total peripheral resistance decreased with both drugs. The sponsor's summary is shown below.



LVEDP was increased over control following clevidipine and decreased following SNP. The same was true of dP/dt and dF/dt.
There was a slight increase in PQ interval in both drug-treated groups.There was no discernible effect on either QRS or uncorrected QT.

Following vagal stimulation, HR response showed a dose-related decrease in the clevidipine animals but not the SNP group. This suggests that clevidipine interferes with the effects of parasympathetic stimulation.

Table V - Effects of vagal nerve stimulation

	Heart rate response			
	Control Δ bpm	Dose I % of control	Dose II % of control	Dose III % of control
Vehicle	-29±10	117±18	130±28	114±46
H 324/38	-39± 6	94±12 *	86±19 *	75±15
Nitroprusside	-41± 5	92±19 *	87±22 *	102±24

Control values are presented as absolute change (Δ) in heart rate, beats per min (bpm) from prestimulatory value. Drug effects are expressed as a percentage of the respective control value. Mean ±SD. Individual values are given in Appendices 13 and 14.
* = p<0.017 from vehicle control in corresponding dose

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Following stimulation of the sympathetic nerves and therefore the cardiac β-adrenergic receptors, HR still declined after each dose of the test drugs and the vehicle.

Table VI - Effects of sympathetic nerve stimulation

	Heart rate response			
	Control Δ bpm	Dose I % of control	Dose II % of control	Dose III % of control
Vehicle	-29±10	86±11	79±20	79±17
H 324/38	-39± 6	83±23	76±14	61±16
Nitroprusside	-41± 5	94±17	89±29	76±37

Control values are presented as absolute change in heart rate beats per min (bpm) from prestimulatory value. Drug effects are expressed as a percentage of respective control values. Mean ±SD. Individual values are given in Appendices 13 and 15.

In bilateral carotid artery occlusion, baroreceptors in the carotid sinus fire, leading to reflex sympathetic activation of the heart and vascular smooth muscles.
Both SNP and clevidipine decreased the vascular response to baroreflex activation during carotid artery occlusion. The sponsor reports this as an action on the vascular smooth muscles rather than impairment of autonomic control.

Table VII - Effects on response to carotid artery occlusion

	Mean arterial pressure (% of control response)			Total peripheral resistance (% of control response)		
	Dose I	Dose II	Dose III	Dose I	Dose II	Dose III
Vehicle	116±24	116±31	128±41	128±34	138±48	146±51
H 324/38	82±21*	63±23*	41±11*	51±21*	28±20 *	28±18*†
Nitroprusside	74±26*	65±31*	36±29*	95±44	94±56*	45±29*

Mean± SD. Individual values are given in Appendix 16a and b.
* = p<0.017 from vehicle in corresponding doses.

Because the preganglionic cardiac sympathetic nerves and vagal nerves were cut in this preparation, only the reflex action of vascular smooth muscles can be evaluated.

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Using blood samples taken during this study, the sponsor constructed the following graphical representation of concentration versus effect.

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Fig 4. Relation between blood concentration and effect during steady state infusion in 6 dogs. E_{max} model was fitted to all individual observed concentration effect data using An PCNONLIN (SCI Software, Kentucky). The data were weighted as $1/Y_{calc}$. Individual values can be found in Appendices 10 and 4.

The sponsor's succinct summary of hemodynamic effects is shown below.

> H 324/38 reduces mean aortic blood pressure, due to a reduction in total peripheral resistance. Cardiac output increased, reflecting an increased stroke volume, as heart rate was kept constant by electrical pacing. The increased stroke volume may be the result of the reduced afterload, facilitating ventricular ejection. Spontaneous heart rate was unaffected by H 324/38 in the doses tested. This was also the case for the electrophysiological parameters studied. Thus, despite relaxation of vascular smooth muscle resulting in up to 40 per cent reduction in total peripheral resistance, H 324/38 had no negative chronotropic or dromotropic effects. Although the inotropic effects are difficult to judge from dP/dt in this study, the present findings of lack of cardiac effects of H 324/38 confirm previous findings in vitro that H 324/38 has a high vascular versus myocardial selectivity (9).

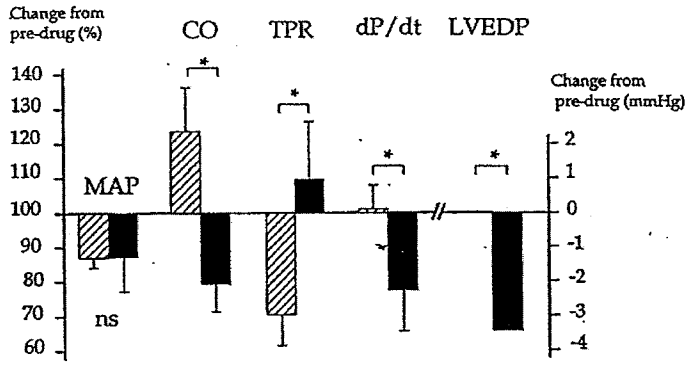

Fig 5. Effects, percentage of control of H 324/38 (hatched bars), 6 nmol/kg/min, and of nitroprusside (black bars), 7 nmol/kg/min on arterial haemodynamics (see Appendix 10). Effects on LVEDP are expressed as change in absolute values (mmHg) from control (Appendix 11c). Abbreviations are given in Appendix 3. * = p<0.017 between groups.

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2.6.2.4 Safety pharmacology

Neurological effects: Overt behavioral effects were not specifically studied. There was one in vitro study to examine effects of clevidipine on nerve transmission.

Cardiovascular effects: 2220-0840-01/50194 Anesthetized Beagles of both sexes were given IV clevidipine after sectioning of the left and right stellate ganglia. All branches except for the cardiac sympathetic trunk were cut. Bilateral vagotomy was also performed. There was a slight increase in PQ interval but no discernible effects on either QRS or uncorrected QT. In humans, this drug has been shown to decrease the QTc.

Pulmonary effects: The closest the sponsor came to this was examining arterial blood gas and pH from anesthetized SHR rats.

Renal effects: *report 108/study 409:* Rats given 2, 30 and 90 nmol/kg/min IV clevidipine or SNP for 40 minutes. Doses ≥30 nmol/kg/min caused decreases of blood pressure up to 50% of control. See below as to decreases in renal blood flow.
LD: An increase in urine flow of 18% ($p<0.05$) was seen. Sodium excretion increased by 27% over control and potassium excretion increased by 22% compared to the control. The changes at

the MD and HD were associated with profound decreases in blood pressure and must be interpreted in that light.

Report 130/study 409: LD did not affect renal blood flow. The MD and HD caused decreases in renal blood flow of 25 and 45 % respectively.

Gastrointestinal effects: dose related inhibition of gastric emptying and propulsive small intestinal movements (5-24%, $p<0.05$ at 24%).

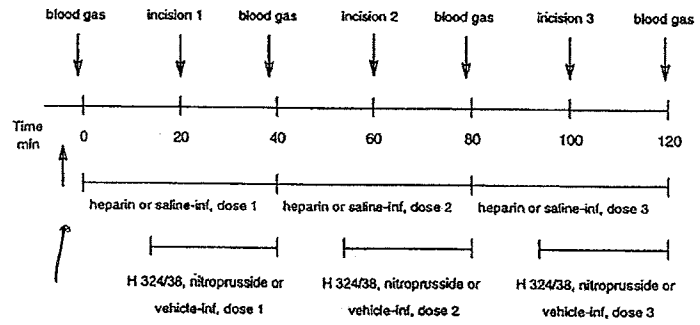
Abuse liability: not done

Other: *report 2220-0388-00/study 21369 Bleeding time determined after cutaneous incisions in the tail of the anesthetized rat*
SHR were given vehicle (— ethanol/saline), heparin (positive control), sodium nitroprusside (blood pressure control)

Study groups, doses and time schedule

Group	Drugs	Bolus	Infusion	Infusion time according to time schedule, min
I	vehicle	-	-	
II	H 324/38 dose 1	-	3 nmol·kg^{-1}·h^{-1}	15-40
II	H 324/38 dose 2	-	10 nmol·kg^{-1}·h^{-1}	55-80
II	H 324/38 dose 3	-	30 nmol·kg^{-1}·h^{-1}	95-120
III	Nitroprusside dose 1	-	3 nmol·kg^{-1}·h^{-1}	15-40
III	Nitroprusside dose 2	-	10 nmol·kg^{-1}·h^{-1}	55-80
III	Nitroprusside dose 3	-	30 nmol·kg^{-1}·h^{-1}	95-120
IV	heparin dose 1	20 IE·kg^{-1}	35 IE·kg^{-1}·h^{-1}	0-40
IV	heparin dose 2	66 IE·kg^{-1}	115.5 IE·kg^{-1}·h^{-1}	41-80
IV	heparin dose 3	200 IE·kg^{-1}	350 IE·kg^{-1}·h^{-1}	81-120

A schematic representation of the protocol.



There were no significant increases in bleeding time with either clevidipine or sodium nitroprusside. Heparin produced a robust and durable increase in bleeding time. Clevidipine did significantly shorten the bleeding time ($p<0.05$) with doses 2 and 3.

Bleeding time		Vehicle Group I	H 324/38 Group II	nitroprusside Group III	heparin Group IV
Dose 1	mean	2.43	2.45	2.43	3.33 *
	SD	0.49	0.51	0.65	0.76
	SEM	0.15	0.16	0.20	0.24
Dose 2	mean	2.25	1.68 †	1.58 †	3.98 *
	SD	0.54	0.42	0.68	1.26
	SEM	0.17	0.13	0.21	0.40
Dose 3	mean	2.50	2.00 †	1.83 †	15.35 *†‡
	SD	1.09	0.50	0.61	5.36
	SEM	0.34	0.16	0.19	1.69

* $p<0.05$ from vehicle control at corresponding dose.
† $p<0.05$ from dose 1 in same group.
‡ $p<0.05$ from dose 2 in same group.

Was the bleeding time decreased because of the dose-dependent decrease in blood pressure during the infusions of SNP and clevidipine (up to 48% decrease in arterial pressure)?

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Clevidipine is highly protein bound (>99.5%) in all species tested. This is summarized in the reproduction of the sponsor's table shown below.

Sponsor's summary of plasma protein binding

Species	Concentration of clevidipine tested		% bound
Rat	100 nM		98.5
Rabbit	100 nM		99.78
Dog	100 nM		99.77
Pig	100 nM		99.52
Human: male*	25-250 nM		99.69-99.75
Human: female*	25-250 nM		99.64-99.67
	Compound tested	Concentration tested	
Human: male and female	H190/90	100nM	99.57
Human: male and female	H190/91	100nM	99.68

*25nM=99.75%

Given intravenously, absorption is assumed to be complete. The sponsor states that the drug is not widely distributed, with a volume of distribution at steady state of 0.45 L/kg in the rat, 0.17 L/kg in the rabbit and 2 L/kg in the dog. However, the radio-label distribution study showed rapid and widespread distribution of drug-associated radioactivity and persistence of the radio-labeled portion of the molecule in the various tissues sampled. The sponsor's summary statement that radioactivity is completely eliminated within 8 days is simply not consistent with all the data.

Metabolites were reported to be formed by 4 main processes:
ester hydrolysis
oxidation of the dihydropyridine ring to the corresponding pyridine
acyl-glucuronidation
decarboxylation

M1 (H152/81), the primary metabolite is produced in a 1:1 ratio with the parent drug. The conversion of occurs by ester hydrolysis. This was the major metabolite found in all species tested. Based on the structure, I would also expect formaldehyde to be produced in the same ratio. The sponsor has only provided a theoretical calculation to address levels of formaldehyde produced.

Summary of major metabolites

species	matrix	Main metabolite
Rat	blood	M1
Dog	blood	M1
Human	blood	M1

Summary of major metabolites

species	matrix	Main metabolite
Rat	Urine	M2 (oxidation of M1 to a pyridine)
Dog	Urine	M3 (acyl-glucuronidation of M1)
Human	Urine	M3 (acyl-glucuronidation of M1)

Summary of major metabolites

species	matrix	Main metabolite
Rat	Feces	M5 decarboxylated pyridine
Dog	Feces	M5 decarboxylated pyridine
Human	Feces	M5 decarboxylated pyridine

Using human hepatocytes from several donors, Clevidipine was found to be a statistically significant inducer of CYP3A4 but not CYP1A2 or 2C9 at the 10 μM (1.2fold induction) and 100 μM (7.3fold induction) concentrations of clevidipine tested. The M1 metabolite was also used for the induction studies and also produced induction of CYP3A4 of ~8.7 fold at a concentration of 100 μM.

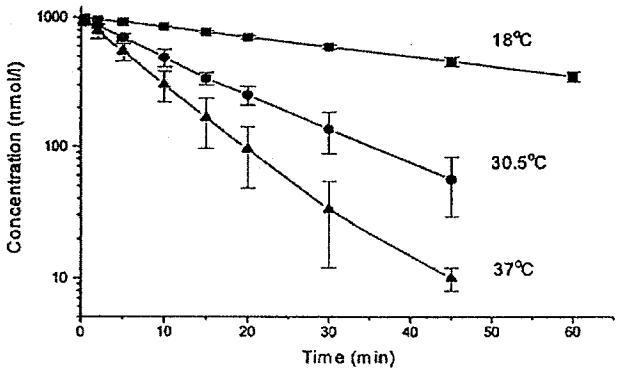
Excretion was studied following IV administration by collecting rat urine and feces for 7 days. Most of the radioactivity was excreted during the first 72 hours. In both male and female rats the majority of excretion appeared to be in the feces.

Species	Dose (μmol/kg)	Sex	% of administered dose of radioactivity excreted in the matrix		Total recovered
			urine	feces	
Rat (n=8)	3.6	m/f	35	56	91
Dog (n=8)	4.0	m/f	21	64	85
Human (n=8)	0.72	m	68	15	83

There was no apparent difference in the excretion patterns between male and female rats and dogs.

The effects of temperature on hydrolysis were examined at 18°, 30.5°, and 37°C. The half-life of clevidipine increased with decreasing temperature. The mean half-life was reported as 6 minutes at 37°C, 11 minutes at 30.5°C and 40 minutes at 18°C. This was reported as consistent with the in vivo effects seen in the hypothermia of cardio-pulmonary by-pass.

Mean ± SD concentrations of H 324/38 in blood at different temperatures (n=4).



2.6.4.2 Methods of Analysis

Report BA251 Methods of analysis for dog plasma samples (report 1312-225) SDS is added to the samples as an esterase inhibitor to prevent metabolism of clevidipine. Ascorbic acid prevents oxidation of the M1 metabolite. The recovery of clevidipine was ~86% (CV 3.0) with a LOQ of 50 nmol/l.

Report41312-0180-01(report1365)Validation for determination of clevidipine in rabbit plasma using analytical methods BA-272 and BA286
GC-MS methods and fluorescence detection.

Stability in solution: in methanol, stable at least 6 months at -18°C
H152/81 stable for at least 3 months
In blood with added SDS: no degradation of Clevidipine found at 4 hours room temperature or at 12 months when stored at -70°C.
H152/81 showed low stability in blood/SDS at room temperature.
Addition of ascorbic acid improved stability to 1 hour at room Temperature and at -70°C samples could be stored for up to 5 Months.
Absolute recovery of clevidipine from SDS-blood was 92.8%, 86% and 84% at 125, 31 and 1.2 nmol/l respectively. Absolute recovery of the M1 metabolite was 85%.

Linearity(<15% from nominal value) was found in 2.5-350 nmol/l . For the metabolite linearity was found from 50-30000nmol/l.

2.6.4.3 Absorption

Report 2131-0122/Study2131-150 PK of H324/38 in male and female rats after constant rate infusion at 3 different doses Drug was infused into the jugular vein of anesthetized rats at a constant rate for 120 minutes. Target doses were 20, 67 and 200 nmol/kg/min. Actual concentrations of drug were found to be 99,94 and 94% of the target. Blood samples were collected from an arterial cannula at 30,60,90,120 sec., 110 and 115 min during the infusion and 15, 30, 45, 60sec and 5 and 20 minutes after stopping the infusion. Blood concentration of H324/38 was determined by GC/MS.

AUCα = the area under the blood concentration vs time, associated with the initial rapidly declining portion of the curve.
Vc= volume of the central compartment
T1/2α= half life during the α elmination

Infusion rate (nmol/kg/min)	T1/2α (sec)	Cl (l/min/kg)	Vc (l/kg)	Vss (l/kg)	Css (nmol/l)	AUCα (%)
20	18±11	0.35±0.07	0.18±0.09	1.43±1.30	58.9±11.1	82±13
67	16±7	0.30±0.07	0.12±0.03	0.42±0.22	223±57	82±5
200	19±6	0.35±0.07	0.20±0.07	0.46±0.19	556±95	79±17

Under the conditions of the study there was no apparent difference in PK parameters between the sexes.

Report1365/Study 23950 PK of clevidipine in female Dutch rabbits following continuous IV infusions at 2 different doses Female Dutch rabbits were infused for 30 minutes with 54 and83 mol/kg/min for a total infusion time of 1 hour. Blood samples were collected during the infusion at 10, 20, 28, 40, 50 and 58 minutes after the start. Additional blood samples were collected at unspecified times after stopping the infusion.

The text of the report notes that one rabbit had clevidipine in a pre-test blood sample,the reason for which was unknown. The blood flow of a rabbit was listed as 0.07l/min/kg. There is no explanation why the samples from two different infusion rates were apparently pooled.

Table 3. Pharmacokinetic parameters of clevidipine obtained from pooled blood concentrations during infusion of 54 nmol/min/kg and 83 nmol/min/kg for 30 min. each and following termination of the highest dose rate.

CL L/min/kg	V_c L/kg	V_{ss} L/kg	C_1^*	C_2^*	$t_{1/2\lambda1}$ sec	$t_{1/2\lambda2}$ min
0.314	0.10	0.17	0.996	0.004	13	3.2

* expressed as fraction of the intercept. When different dose rates are administered, the computer program converts the first dose rate to an I.v bolus dose and reports the intercept for this dose rate.

Under the conditions of the study the half life was extremely short with a limited volume of distribution.

Report 1312-225/Study 265/519 TK in 4 week intravenous infusion (12 hours/day) study in the Beagle

Blood concentrations of clevidipine and M1 were determined by GCMS and LC in blood samples taken on day 1, day 7 and day 28 of dosing from the 3 male and 3 female dogs in each of 6 groups. The dogs were treated daily with 0 (2 groups), 15, 35, 70 and 145μmol/kg for 12 hours a day. On each of the sampling days, the clevidipine animals were sampled before the infusion and 1,6 and 12 hours after the start of infusion. From group 4 (35μmol/kg), samples were also taken after the daily 12 hours of infusion on day 1 and day 28 at 20 and 40 seconds, 1,2,4,6,15 and 30 minutes after the end of infusion. Control groups were sampled only once a day, 6 hours after start of infusion.

The results presented are almost solely individual animal blood levels. It is stated in the text that there were no differences between the sexes in blood levels of parent drug or metabolite. There seems to be an increase in steady state levels with increasing dose. The calculated group TK parameters were presented for only 1 group and are shown below.

Values provided were geometric mean and range

T1/2α min	T1/2β min	Vc l/kg	Cl l/min/kg	Vssl/kg	AUCα
Day 1					
0.73	13.1	0.62	0.512	1.98	85
Day 28					
1.35	16.4	0.53	0.253	1.22	82

There was decrease in clearance from day 1 to day 28 at the dose studied.

The metabolite had a much greater persistence in the blood. There was also a profound increase in t1/2 from day 1 to day 28 in 2 animals and a decrease in 1 animal. This was unexplained in the report.

INDIVIDUAL HALF-LIVES OF H 152/81 CALCULATED FROM POST-INFUSION BLOOD LEVELS ON DAYS 1 AND 28, STUDY No. 265/519

Group: 4
Daily dose: 35 µmol/kg, intravenous infusion during 12 hours

Dog No	Sex	Day 1 $t_{½}$ (min)	Day 28 $t_{½}$ (min)
269	M	24	21
270	M	25	23
271	M	25	19
272	F	33	83
273	F	20	132
274	F	48	28

2.6.4.4 Distribution

ReportT2877Whole-Body autoradiographic study on the distribution of radioactivity in rats after single intravenous infusion of H324/38-^{3}H

A single dose of 0.78µmol/kg was given by IV infusion to male Lister Black Hooded rats and to pregnant (GD20) rats in a 5 minute/animal interval. The drug was dissolved in Intralipid®.

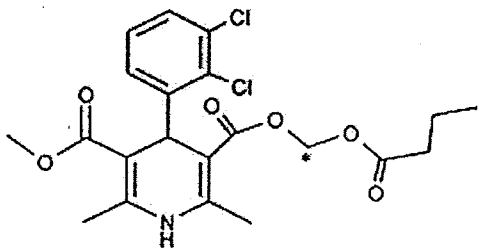
1 minute after completed infusion in male rat: High levels of radioactivity seen in blood, brown fat, myocardium, diaphragm, tongue, lung, dental pulp, exorbital lacrimal gland, choroid plexus, adrenal and pigmented layer of eye.

After infusion to pregnant rats, high levels of radioactivity were seen in the blood and amniotic membrane up to 15 mintues and in the ovary and placenta up to 5 minutes. Low levels of radioactivity were seen in the fetus for the 4 hours of investigation.

Drug-associated radioactivity was retained in the connective tissues for 1-2 days. H324/38 also crossed the blood brain barrier and the placenta. Retention in melanin was not detected after 2 days. The test compound was reported as completely excreted via the bile and urine. Two days after dosing the urine still showed very high levels of radioactivity. By 8 days after infusion, no radioactivity was reported for any organ or tissue.

Report PK04-095 Tissue distribution of ^{14}C-clevidipine in male Sprague-Dawley and Long Evans rats following single intravenous bolus dose

A single intravenous 5 mg/kg dose of ^{14}C-clevidipine was given to each of 7 male SD and 3 male LE rats. The distribution of radioactivity was determined by quantitative whole body autoradiography(QWBA). Concentrations of drug-derived radioactivity were determined in blood, plasma, tissues and bodily fluids to evaluate tissue distribution and elimination of radioactivity. Total ^{14}C-clevidipine-derived radioactivity per organ or tissue was calculated for a limited list of organs based on standard organ weights from the literature and reported as a percent of the administered dose. The LOQ for QWBA was 0.96 nCi/g and the upper limit was 8542nCi/g.



Clevidipine

* designates ^{14}C radiolabel position

The reviewer's informal consultation with the CDER chemists confirmed that the radio-label would go with the formaldehyde moiety.

Widespread distribution of radioactivity occurred with maximum observed concentrations in tissues reaching 0.5-8 hours post-dose. Maximum observed blood and plasma concentrations were seen at 2 hours post-dose. The ratio of drug in blood to plasma showed a shift from plasma to blood cells over 72 hours. A similar shift was reported for the pigmented rats.

Table 2a. Concentration of Radioactivity in Blood and Plasma at Specified Times Postdose in Male Albino Rats Following a Single Intravenous Dose of ^{14}C-Clevidipine

Time (h)	Animal Number	Concentration (µg equiv/g)			
		QWBA-Derived	LSC-Derived		
		Blood	Blood	Plasma	B/P Ratio
0.5	1-0.5				0.619
1	1-1				0.644
2	1-2				0.611
4	1-4				0.586
8	1-8				0.619
24	1-24				0.906
72	1-72				1.449

BLQ: Below the limit of quantitation (QWBA blood = 0.063 µg equiv/g; LSC blood = 0.003 µg equiv/g; and LSC plasma = 0.002 µg equiv/g).

Within the first half hour, the highest values of radioactivity were seen in the pancreas, thyroid and bone marrow of albino rats. High levels were also seen in the bone marrow and thyroid of pigmented rats.

Table 3. Concentration of Radioactivity in Tissues and Fluids at Specified Times Postdose in Male Albino Rats Following a Single Intravenous Dose of ^{14}C-Clevidipine

Tissue Type	Tissue	Concentration (µg equiv/g) Animal Number/Time Point						
		1-0.5 0.5 h	1-1 1 h	1-2 2 h	1-4 4 h	1-8 8 h	1-24 24 h	1-72 72 h
Vascular/Lymphatic	Aorta							
	Blood							
	Bone marrow							
	Lymph nodes							
	Spleen							
Excretory/Metabolic	Bile							
	Kidney							
	Liver							
	Renal cortex							
	Renal medulla							
	Urinary bladder							
	Urine							
Central Nervous System	Cerebellum							
	Cerebrum							
	Choroid plexus							
	Medulla							
	Pineal gland							
	Spinal cord							
Endocrine	Adrenal gland							
	Pituitary gland							
	Thymus							
	Thyroid							

Table 3. cont. Concentration of Radioactivity in Tissues and Fluids at Specified Times Postdose in Male Albino Rats Following a Single Intravenous Dose of ^{14}C-Clevidipine

Tissue Type	Tissue	Concentration (µg equiv/g) Animal Number/Time Point 1-0.5 0.5 h	1-1 1 h	1-2 2 h	1-4 4 h	1-8 8 h	1-24 24 h	1-72 72 h
Secretory	Exorbital lacrimal gland							
	Harderian gland							
	Intra-orbital lacrimal gland							
	Pancreas							
	Parotid gland							
	Salivary gland							
Fatty	Fat (abdominal)							
	Fat (brown)							
Dermal	Non-pigmented skin							
	Pigmented skin							
Reproductive	Bulboglandular muscle							
	Epididymis							
	Preputial gland							
	Prostate							
	Seminal vesicle							
	Testes							
Skeletal/Muscular	Bone							
	Diaphragm							
	Incisor pulp							
	Muscle							
	Myocardium							

Table 3. cont. Concentration of Radioactivity in Tissues and Fluids at Specified Times Postdose in Male Albino Rats Following a Single Intravenous Dose of ^{14}C-Clevidipine

Tissue Type	Tissue	Concentration (µg equiv/g) Animal Number/Time Point 1-0.5 0.5 h	1-1 1 h	1-2 2 h	1-4 4 h	1-8 8 h	1-24 24 h	1-72 72 h
Respiratory Tract	Lung							
	Nasal turbinates							
	Trachea							
Alimentary Canal	Buccal mucosa							
	Cecum							
	Cecum contents							
	Esophageal contents							
	Esophagus							
	Gastric mucosa							
	Large intestinal contents							
	Large intestine							
	Small intestinal contents							
	Small intestine							
	Stomach							
	Stomach contents							
Ocular	Ciliary body/processes							
	Cornea							
	Eye							
	Lens							
	Iris							
	Retina/choroid							

Radioactivity was measured in the testes, accessory sex organs and the eyes for at least 672 hours in the male pigmented rats. Radioactivity in the CNS was low but quantifiable through 672 hours after dosing. Drug associated radioactivity was associated with the melanin-containing tissues in the eye and skin. The drug-derived radioactivity was consistently higher in the pigmented vs the non-pigmented skin.

Table 6. Concentration of Radioactivity in Tissues and Fluids at Specified Times Postdose in Male Pigmented Rats Following a Single Intravenous Dose of ^{14}C-Clevidipine

		Concentration (µg equiv/g)		
		Animal Number/Time Point		
Tissue Type	Tissue	2-24 24 h	2-336 336 h	2-672 672 h
Vascular/Lymphatic	Aorta			
	Blood			
	Bone marrow			
	Lymph nodes			
	Spleen			
Excretory/Metabolic	Bile			
	Kidney			
	Liver			
	Renal cortex			
	Renal medulla			
	Urinary bladder			
	Urine			
Central Nervous System	Cerebellum			
	Cerebrum			
	Choroid plexus			
	Medulla			
	Pineal gland			
	Spinal cord			
Endocrine	Adrenal gland			
	Pituitary gland			
	Thymus			
	Thyroid			
Secretory	Exorbital lacrimal gland			
	Harderian gland			
	Intra-orbital lacrimal gland			
	Pancreas			
	Parotid gland			
	Salivary gland			
Fatty	Fat (abdominal)			
	Fat (brown)			
Dermal	Non-pigmented skin			
	Pigmented skin			

Table 7. Recovery of Radioactivity (% of Dose) in Various Tissues at Specified Times Postdose in Male Pigmented Rats Following a Single Intravenous Dose of ^{14}C-Clevidipine

Tissue		Standard Rat Tissue Weights (g) (250 g animal)	% of Dose[a] Animal Number/Time Point 2-24 24 h	2-336 336 h	2-672 672 h
Adrenal gland	(ref b)	0.05			
Brain[c]	(ref b)	1.8			
Myocardium	(ref b)	1.0			
Kidney	(ref b)	2.0			
Liver	(ref b)	10.0			
Lung	(ref b)	1.5			
Spleen	(ref b)	0.75			
Muscle	(ref d)	45.5% of body weight			
Skin[c]	(ref d)	18.0% of body weight			
Stomach	(ref d)	0.50% of body weight			
Total % of Dose			5.45	1.07	0.38

[a] % of dose corrected for actual animal weight.

Table 4. Recovery of Radioactivity (% of Dose) in Various Tissues at Specified Times Postdose in Male Albino Rats Following a Single Intravenous Dose of ^{14}C-Clevidipine

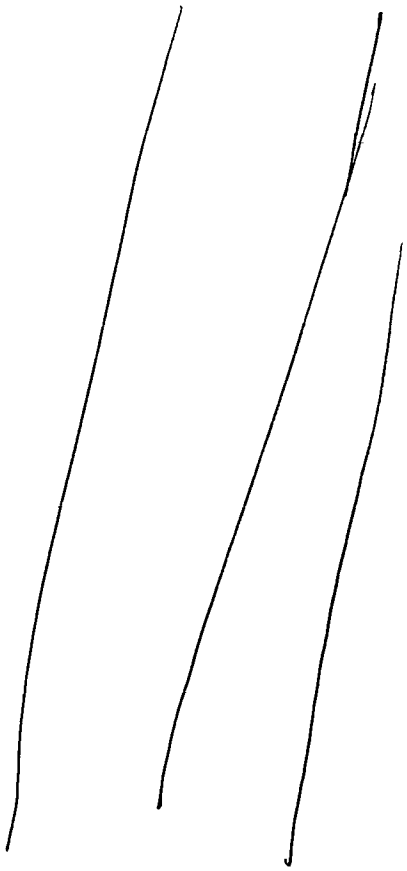
Tissue		Standard Rat Tissue Weights (g) (250 g animal)	% of Dose[a] Animal Number/Time Point 1-0.5 0.5 h	1-1 1 h	1-2 2 h	1-4 4 h	1-8 8 h	1-24 24 h	1-72 72 h
Adrenal gland	(ref b)	0.05							
Brain[c]	(ref b)	1.8							
Myocardium	(ref b)	1.0							
Kidney	(ref b)	2.0							
Liver	(ref b)	10.0							
Lung	(ref b)	1.5							
Spleen	(ref b)	0.75							
Muscle	(ref d)	45.5% of body weight							
Skin	(ref d)	18.0% of body weight							
Stomach	(ref d)	0.50% of body weight							
Total % of Dose			9.24	9.11	7.37	7.96	7.28	4.16	2.43

[a] % of dose corrected for actual animal weight.

Something of interest is the increase in tissue: plasma ratio of radioactivity seen in some tissues following a single dose. One possible interpretation is that the radioactivity is cleared from plasma and tissue at different rates.

Table 8. Tissue:Plasma Ratio of Radioactivity in Tissues and Fluids at Specified Times Postdose in Male Pigmented Rats Following a Single Intravenous Dose of ^{14}C-Clevidipine

		Tissue/Plasma Ratio		
		Animal Number/Time Point		
Tissue Type	Tissue	2-24 24 h	2-336 336 h	2-672 672 h
Vascular/Lymphatic	Aorta			
	Blood			
	Bone marrow			
	Lymph nodes			
	Spleen			
Excretory/Metabolic	Bile			
	Kidney			
	Liver			
	Renal cortex			
	Renal medulla			
	Urinary bladder			
	Urine			
Central Nervous System	Cerebellum			
	Cerebrum			
	Choroid plexus			
	Medulla			
	Pineal gland			
	Spinal cord			
Endocrine	Adrenal gland			
	Pituitary gland			
	Thymus			
	Thyroid			
Secretory	Exorbital lacrimal gland			
	Harderian gland			
	Intra-orbital lacrimal gland			
	Pancreas			
	Parotid gland			
	Salivary gland			
Fatty	Fat (abdominal)			
	Fat (brown)			
Dermal	Nonpigmented skin			
	Pigmented skin			



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There is also an increase in tissue: plasma ratio of radioactivity in the reproductive tract.

Tissue Type	Tissue	Tissue/Plasma Ratio Animal Number/Time Point 2-24 24 h	 2-336 336 h	 2-672 672 h
Reproductive	Bulboglandular muscle			
	Epididymis			
	Preputial gland			
	Prostate			
	Seminal vesicle			
	Testes			
Skeletal/Muscular	Bone			
	Diaphragm			
	Incisor pulp			
	Muscle			
	Myocardium			
Respiratory Tract	Lung			
	Nasal turbinates			
	Trachea			
Alimentary Canal	Buccal mucosa			
	Cecum			
	Cecum contents			
	Esophageal contents			
	Esophagus			
	Gastric mucosa			
	Large intestinal contents			
	Large intestine			
	Small intestinal contents			
	Small intestine			
	Stomach			
	Stomach contents			
Ocular	Ciliary body/processes			
	Cornea			
	Eye			
	Lens			
	Iris			
	Retina/choroid			

Does the formaldehyde persist in the tissues as formaldehyde (cross-linking proteins?) or is the carbon incorporated into the one-carbon pool and from there incorporated into macromolecules?

Report 1327/23959 In vitro protein binding of clevidipine and its enantiomers in plasma from different animal species and humans

A liquid chromatography method was used for plasma protein binding studies, using felodipine as a reference compound. It was stated that the rapid hydrolysis of clevidipine in blood and plasma, made ultra filtration and equilibrium dialysis unsuitable methodology. Unlabelled and tritium labeled clevidipine were used. Plasma was collected from 6 humans (3m, 3f), 3 male rats, 3 dogs(1m, 2f). 3 rabbits(2m, 1f) and 3 female pigs.

An aliquot of diluted plasma was injected onto the column and the extent of drug bound to the plasma proteins was detected as a peak on the chromatogram. To examine linearity, different volumes (50, 100, 150 and 200 µl) of diluted plasma were injected onto the column. Percentage bound to plasma proteins was calculated as

%bound= (1- Cu/(Cu+Cb))x100 where Cu= unbound concentration and Cb = bound concentration corrected for dilution.

Clevidipine and the enantiomers were > 99.5% protein bound in each of the species tested. The protein binding was constant in the studied concentration range of 25-250 nM. The free fractions of the enantiomers in human plasma were 0.43(H190/90) and 0.32% (H190/91).

2.6.4.5 Metabolism

Report1169/study 23652 Determination of the in vitro half-life of clevidipine in human blood at different temperatures and in diluted blood.

The experiments were conducted at 37, 30.5 and 18°C with blood diluted 1:2 with Ringer-glucose solution at 30.5°C.

The blood concentration of parent drug declined monoexponentially vs time for approximately 90 % of the added amount of clevidipine at 37and 30.5°C and over a period of 60 minutes at 18°C. Mean half-life was 6 minutes at 37°C,
11 minutes at 30.5°C, and 40 minutes at 18°C.
In blood diluted with an equal volume of Ringer-glucose solution, the mean half-life was 11±2 minutes at 30.5°C. The hydrolysis rate was similar in the samples from the 2 m and 2 f donors.

Report 1253/study 23741Determination of the in vitro half-life of clevidipine in blood and plasma from subjects with phenotypically abnormal plasma pseudocholinesterase activity

Studies were conducted at 37°C.
The mean half life was 9.3±1.7 minutes in blood from subjects homozygous for the atypical plasma cholinesterase gene, 8.0±0.8 min in blood from subjects heterozygous for the atypical gene and 5.8±1.1 min in blood from subjects with normal plasma cholinesterase activity (control).

Summary of in vitro hydrolysis of clevidipine

	Mean in vitro half life	
	blood	plasma
Homozygous for atypical plasma cholinesterase (n=3)	9.3±1.7 min	44±12min
Heterozygous for atypical plasma cholinesterase (n=3)	8.0±0.8 min	28±7 min
Normal plasma cholinesterase(control) (n=4)	5.8±1.1 min	31±6 min

Report 1308/1309/Study 142,139 Metabolism of [^{3}H]-labeled clevidipine following intravenous administration to rats and dogs

A single intravenous dose of [^{3}H]-labeled clevidipine was given to male and female rats and dogs. The target doses were 3.6 μmol/kg for rats and 4 μmol/kg for dogs. Urine and feces were collected for 7 days in the rat and for 5 days in the dog after dosing. Reversed phase LC with on-line radioactivity detection was used for analysis. The urine and feces were taken from mass balance studies (2131-0116 and 2131-0121). Both species had 4animals/sex (n=8 per species).

No parent compound was detected in the urine or feces of either species. No qualitative differences in metabolic pattern were reported for the urine or feces of the 2 species and the 2 sexes.

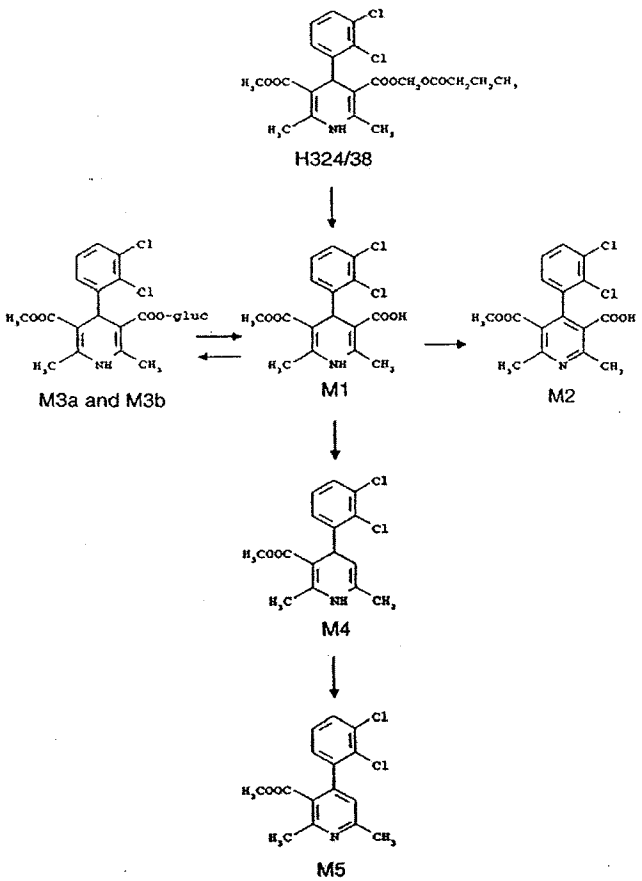
Metabolites were identified by LC retention times in comparison to synthetic references. The metabolites were formed by 4 main routes of metabolism: ester hydrolysis, oxidation of the dihydropyridine ring to the corresponding pyridine, acyl-glucuronidation and decarboxylation. Clevidipine was completely metabolized to the primary metabolite M1. Oxidation of M1 gave the M2 pyridine, the main urinary metabolite in the rat. The main urinary metabolite in the dog was the M3 acyl-glucuronide (which separated into diastereomers) of M1. The decarboxylated pyridine M5 was the primary fecal metabolite in both species.

Recovery as a percent of the dose given

	recovery of dose		
	Total recovery	Recovery in urine	Recovery in feces
Rat	88-101%	22-43%	45-68%
Dog	82-88%	12-42%	42-73%

Both the renal and fecal routes of excretion were important in the rat and dog.

FIGURE 6. Proposed metabolic pathways of clevidipine in the rat and dog.



Report 2131-0116/Study 2131-139 Mass balance and metabolic fate of H324/38-^{3}H in the rat after intravenous administration

Radio-labeled clevidipine was administered intravenously to 4 f and 4 m Sprague Dawley rats at a target dose of 3.6μmol/kg. Urine and feces were collected for 7 days after dosing.

Most of the radioactivity was excreted in the first 72 hours after dosing. Approximately 45-68% of the radioactivity was excreted in the feces, indicating biliary excretion. Recovery in the urine was 22-43% of the dose. The results of this study were reported in report 1308/1309.

There was only a trace amount of radioactivity associated with parent compound, indicating extensive metabolism. There were no apparent differences in the excretion pattern of radioactivity between male and female rats. Approximately 0.3% of the dose was found in the carcass, indicating that most of the dose had been excreted.

Report 2131/0118 /Study 2131-145 Determination of the in vitro half-life of clevidipine in blood from the rat, the dog and man[sic].

Rate of hydrolysis of clevidipine in fresh blood from rats, humans and dogs of both sexes was determined at 37°C. Samples were collected from:

Sprague Dawley rats: 6m, 6f
Beagles: 3m, 3f
Humans: 3m, 3f

Summary of mean half lives

species	Mean half-life
Rat blood	34 sec
Dog blood	16 min
Human blood	6 min

The stability of the drug was not affected by the sex of the blood donor. In all 3 species the drug was reported to be transformed to its primary metabolite at a rate corresponding to the hydrolysis rate of the parent compound. The mean half life showed marked species variability under the conditions of the study.

Report 030508a/study Induction potential of Cytochrome P450 Isoforms CYP1A2, CYP2C9 and CYP3A4 by the test article clevidipine in primary cultured human hepatocytes

Freshly isolated hepatocytes from 5 donors (3 f and 2 m) were plated in collagen I-coated 24-well plates. Hepatocytes were exposed to clevidipine for a total of 3 days. Samples for P450 induction were analyzed by measuring the P450 specific probe substrate catalytic activity using HPLC analysis with absorbance detection.

Final clevidipine concentrations were 1, 10 and 100 µM in DMSO. Each concentration was tested in triplicate. After the final day of exposure the medium was removed and replaced with media containing the P450 probe substrates.

Table 1. Marker enzyme assay parameters

Probe substrate	P450 isoform	Reaction	Assay concentration
Testosterone	CYP3A4	6β-Hydroxylase	200 µM
Phenacetin	CYP1A2	O-Deethylase	100 µM
Diclofenac	CYP2C9	4'-Hydroxylase	100 µM

To test for inhibition of catalytic activity, a fresh set of cells was exposed to a positive control inducer. That media was then removed and replaced with fresh media + clevidipine. Cells were then incubated for 30 minutes, washed and incubated with P450 probe substrate.

Table 3. Positive control inducer chemicals

Enzyme	Positive control inducer	Final concentration	Solvent for delivery
CYP1A2	ß-Naphthoflavone	20 µM	DMSO
CYP2C9	Rifampicin	20 µM	DMSO
CYP3A4	Rifampicin	20 µM	DMSO

Supernatant from the incubations was analyzed by HPLC.

Results

Based on the results presented in tables 4, 5 there was no apparent induction effect of clevidipine on CYP1A2 and CYP2C9. Table 6 showed an induction of CYP3A4.

Table 6. Effect of clevidipine and positive control rifampicin (RIF) on CYP3A4-catalyzed testosterone 6β-hydroxylase and protein content in hepatocytes from 3 donors

Donor	Treatment	Activity (pmol/mg/min)	Fold induction	Protein (mg/well)
HH136	0 μM (DMSO vehicle)	89 ± 4.6	-	0.403 ± 0.002
	1 μM clevidipine	80 ± 7.0	0.90 ± 0.075	0.403 ± 0.017
	10 μM clevidipine	102 ± 4.4	1.2 ± 0.058	0.426 ± 0.019
	100 μM clevidipine	486 ± 27	5.5 ± 0.29	0.457 ± 0.006
	20 μM RIF	1311 ± 29	15 ± 0.58	0.464 ± 0.014
HM017	0 μM (DMSO vehicle)	15 ± 1.7	-	0.314 ± 0.013
	1 μM clevidipine	14 ± 1.2	0.89 ± 0.072	0.319 ± 0.016
	10 μM clevidipine	19 ± 2.3	1.3 ± 0.17	0.331 ± 0.011
	100 μM clevidipine	158 ± 8.4	10 ± 0.58	0.334 ± 0.005
	20 μM RIF	1365 ± 93	91 ± 6.1	0.334 ± 0.006
HH137	0 μM (DMSO vehicle)	5.0 ± 0.42	-	0.406 ± 0.062
	1 μM clevidipine	5.4 ± 0.64	1.1 ± 0.12	0.394 ± 0.016
	10 μM clevidipine	6.0 ± 0.42	1.2 ± 0.10	0.384 ± 0.020
	100 μM clevidipine	31 ± 2.3	6.2 ± 0.49	0.415 ± 0.017
	20 μM RIF	622 ± 62	124 ± 13	0.423 ± 0.016

Data are the mean ± standard deviation from three separate wells in each group.

The sponsor summarized the results as follows:

Table 10. Inhibitory effect of clevidipine at 100 μM on CYP1A2, 2C9 and 3A4 activity in hepatocytes from three donors hepatocytes

Donors	CYP 1A2 Inhibition (%)	CYP 2C9 Inhibition (%)	CYP 3A4 Inhibition (%)
*1	24	82	0.4
2	3.6	0.57	13
3	-4.8	3.0	12

Where does the inhibition come from? The textual summary of the report noted that "Clevidipine was found to be a statistically significant inducer of CYP3A4 but not CYP1A2 or 2C9 at the 10 μM and 100 μM concentrations of clevidipine tested. "

Report 030508d/study Induction potential of Cytochrome P450 isoforms CYP1A2, CYP2C9 and CYP 3A4 by the primary metabolite (H152/81) of clevidipine in primary cultured human hepatocytes

The methods for this study were the same as those in the preceding report except that the cells were exposed to the M1 metabolite rather than the parent drug.

There does not appear to be any induction effect on CYP1A2. There were some mild inconsistent decreases in CYP2C9.

Table 8. Summary of effect of H152/81 and positive control rifampicin on CYP2C9-catalyzed diclofenac 4'-hydroxylase and protein content in hepatocytes of 3 donors hepatocytes – Means of three donors (HM018, HH137 and HH138)

Treatment	Activity (pmol/mg/min)	Fold induction	Protein (mg/well)
0 μM (DMSO vehicle)	33 ± 23	-	0.331 ± 0.092
1 μM H152/81	31 ± 19	0.97 ± 0.11	0.310 ± 0.068
10 μM H152/81	18 ± 13*	0.54 ± 0.18	0.356 ± 0.030
100 μM H152/81	23 ± 22*	0.63 ± 0.29	0.365 ± 0.050
20 μM RIF	167± 128	4.9 ± 0.76	0.369 ± 0.055

Data are the mean ± standard deviation from three donors in each group. * Significantly different from controls ($p \leq 0.05$)

CYP3A4 showed induction at the 100μM concentration.

Table 9. Summary of effect of H152/81 and positive control rifampicin (RIF) on CYP3A4-catalyzed testosterone 6β-hydroxylase and protein content in hepatocytes of 3 donors hepatocytes – Means of three donors (HH136, HM017 and HH137)

Treatment	Activity (pmol/mg/min)	Fold induction	Protein (mg/well)
0 μM (DMSO vehicle)	39 ± 49	-	0.326 ± 0.047
1 μM H152/81	39 ± 51	0.96 ± 0.037	0.332 ± 0.044
10 μM H152/81	50 ± 65	1.2 ± 0.22	0.337 ± 0.053
100 μM H152/81	294 ± 316*	8.7 ± 5.0	0.350 ± 0.067
20 μM RIF	1235 ± 322	82 ± 60	0.354 ± 0.076

Data are the mean ± standard deviation from three donors in each group. * Significantly different from controls ($p \leq 0.05$)

Report 030512a/Study Inhibition of Cytochrome P4501A2, Cytochrome P4502C9, Cytochrome P4502C19, Cytochrome P4502D6, Cytochrome P4502E1 and Cytochrome P4503A4 catalytic activities by H324/38

Clevidipine was tested for its inhibition potential toward several human CYP 450 isozymes using cDNA expressed enzyme. Incubations were carried out using microsomes from cells expressing the individual human CYP450 enzymes and isozyme specific substrates ± clevidipine. Concentrations of clevidipine required to inhibit 50% of the catalytic activities (IC_{50}) were determined. Final concentrations used were 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 μM.

Positive controls: 7,8-benzoflavone; sulfaphenazole; tranylcypromine; quinidine; 4-methylpyrazole; ketoconazole
The CYP2C19 and CYP2E1 were used in [redacted] preparations.

Summary of findings for H324/38 using cDNA-expressed CYP450

isozyme	Specific substrate	IC50 value(μM)
CYP2C9	Diclofenac4'-hydroxylase	4.4
CYP2C19	[(S)-mephenytoin 4'-hydroxylase	2.5
CYP2D6	Bufuralol 1'-hydroxylase	72
CYP3A4	Testosterone 6β-hydroxylase	8.4

The catalytic activity of CYP1A2 (phenacetin hydoxylase) and CYP2E1(p-nitrophenol hydroxylase) was inhibited by less than 50% at 300μM, the highest concentration of clevidipine assessed.

Report 030512b/ Study 03051b Inhibition of cytochrome P4501A2, Cytochrome P4502C9, Cytochrome P450 2C19, Cytochrome P4502D6, Cytochrome P4502E1, and Cytochrome P4503A4 catalytic activities by H152/81.

The M1 metabolite of clevidipine was tested against cDNA-expressed enzyme of human CYP450 isozymes. Microsomes from cells expressing the individual human cytochrome P450 enzymes were incubated with isozyme specific substrates ± H152/81. The concentration of M1 required to inhibit 50% of the catalytic activities was determined. Concentrations of M1 tested were: 0,0.01, 0.03, 0.1, 0.3, 1,3,10, 30, 100 and 300 μM.

Table 4. Summary of IC_{50} results for H152/81 using cDNA-expressed cytochrome P450s as an enzyme source.

Isoform of Cytochrome P450	IC_{50} (μM)
CYP1A2	>300
CYP2C9	92
CYP2C19	69
CYP2D6	>300
CYP2E1	>300
CYP3A4	198

Based on the preceeding 4 studies, it appears that parent and H152/81 metabolite both have some CYP3A4 induction capability. Under the conditions used, Clevidipine had some inhibitory effect on CYP2C9, 2C19, 2D6 and 3A4.

2.6.4.6 Excretion

Report 2131-0121/Study 2131-142 Mass balance and metabolic fate of H324/38-H^3 in the dog after intravenous administration

Tritium-labeled H324/38 was given intravenously to 4 female and 4 male beagle dogs at a target dose of 4 μmol/kg. Urine and feces were collected for 5 days after dosing.

Amount of radioactivity expressed as percent of the dose excreted in urine and feces after a single IV dose to female dogs

Interval(Hrs)	Dog9-044	Dog 8-108	Dog2-115	Dog2-051	mean±SD
urine					
0-120	19.3	41.9	26.7	15.8	25.9±11.6
feces					
0-120	62.6	41.6	60.6	69.6	58.6±12.0
sum	81.9	83.5	87.3	85.4	84.5±2.3

Amount of radioactivity expressed as percent of the dose excreted in urine and feces after a single IV dose to male dogs

Interval(Hrs)	Dog9-044	Dog 8-108	Dog2-115	Dog2-051	mean±SD
urine					
0-120	12.6	15.9	25.9	11.7	16.5±6.5
feces					
0-120	70.3	71.8	60.3	73.3	68.9±5.9
sum	82.9	87.7	86.2	85.0	85.5±2.0

The majority of radioactivity was excreted within the first 72 hours. There were no consistent differences between the sexes. Up to 35% of the administered dose was excreted in the urine from 0-72 hours. The percent of the dose excreted in the feces was from 31% to 73% from 0-72 hours.

About 12% of the given radioactivity in the feces was detected at the same retention time as the parent drug. There were no reported qualitative differences between the metabolites in urine and feces from the two sexes.

This excretion pattern differs from humans in that :

- **Excretion is more rapid in humans (within first 24 hours)**
- **Major route of excretion in humans is via the urine**

2.6.4.6 Pharmacokinetic drug interactions

Report 1264/Study 23687 The in vitro hydrolysis rate of clevidipine in human blood in the presence of different compounds used in general anesthesia.

The rate of hydrolysis was estimated by measuring the formation rate of the primary metabolite of clevidipine, H152/81(M1).

Drugs used: thiopental sodium, propofol, isoflurane, pancuronium bromide, vecuronium bromide, morphine, fentanyl, diltiazem. At least 3 different concentrations of the potential inhibitor were studied and compared with a control value from the same individual. The lowest concentration tested was selected to be close to therapeutic concentrations. Control formation rate was determined after addition of the same volume of vehicle to the blood instead of the potential inhibitor.

Target concentration of clevidipine was 1 μmol/l in all experiments. All incubations were performed at 37°C. Isoflurane was also incubated at 18°C.

Fresh blood from 4 healthy humans was used in each experiment. The potential inhibitor was added to the incubation mixture. Half of the incubation mixture was used to prepare standards. The remaining half was used for the hydrolysis experiment. Samples were taken 15, 30, 135 and 150 sec after addition of clevidipine. Concentrations of the primary metabolite were performed with LC and fluorescence detection.

Under the conditions of the study, most of the drugs tested did not affect the hydrolysis rate of clevidipine. Fentanyl caused a slight increase in the rate of hydrolysis and diltiazem caused a non-significant decrease.

	NaCl	Fentanyl			NaCl	Diltiazem		
	0.9%	ng/ml blood			0.9%	µg/ml blood		
		2	20	200		0.1	1	10
Mean	103	103	107	114*	109	107	110	93
SD	15	15	20	17	8	14	18	16

*p<0.05

Both pancuronium and vecuronium caused significant reductions in hydrolysis of 21% and 26% respectively.

H 152/81 (pmol/min/g blood)

	NaCl	Pancuronium bromide					
	0.9%	µg/ml blood					
		0.05	0.5	5	50	500	1000
Mean	95	81*	68*	68*	72*	72*	75*
SD	11	7	4	3	6	2	7

*p<0.05

	NaCl	Vecuronium bromide		
	0.9%	µg/ml blood		
		0.35	3.5	35
Mean	103	90*	80*	76*
SD	12	13	11	9

*p<0.05

The clinical significance of this, if any is unclear. However, this does show foresight on the part of the sponsor to make this assessment.

2.6.4.8 Other Pharmacokinetic Studies

Report 1334/Study 23823 Pharmacokinetics of H152/81 in male and female rats after IV bolus injection

The intended dose of the metabolite was 2.25μmol/kg administered into the jugular vein of 4 male and 4 female Sprague Dawley rats. Each dose was injected over a 60 second period. Blood was collected from carotid cannulas at 5, 10, 30, 60 minutes and 2,4,7,12,24 and 31 hours after the bolus administration.

The blood levels of H152/81 declined bi-exponentially after the iv bolus. Mean half-life associated with the terminal phase (t1/2λz) was 12.8 hours in male rats and 18.8 hours in female rats.

	males	Females
$t_{1/2\lambda z}$ hours	12.8	18.8
Mean clearance ml/h/kg	22	12
Vss l/kg	0.38	0.33

Under the conditions of the study, there are sex-related differences in half life and clearance of the M1 metabolite.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: See Overall Toxicology Summary

Genetic toxicology: One of the major metabolites is the genetic toxicant formaldehyde. A summary table of the genetic toxicology is provided in the overall summary.

Carcinogenicity: No carcinogenicity studies as such were performed for clevidipine due to the proposed short term use. However, there is information regarding at least one metabolite. ACGIH lists formaldehyde as a suspected human carcinogen (A2) and EPA calls formaldehyde a probable human carcinogen(B1).

Reproductive toxicology: The effects are typical of those seen with other calcium channel blockers: dystocia, delayed parturition, impaired male fertility. Something that appears to be unique to clevidipine is the finding of unusual or atypical estrous cycle length and pseudopregnancy.

Special toxicology: Local tolerance studies were performed. Under the conditions of the studies, there is little irritation potential.

2.6.6.2 Single-dose toxicity

T2855 Single dose toxicity of clevidipine in rats after IV administration

Doses administered over 1 minute: control(0), 23,33,93, 110, 130, 140, 160 mg/kg.
Animals were observed for 14 days after dosing.
Signs reported for all doses: irregular breathing and decreased motor activity for up to 45 minutes after dosing.
≥93 mg/kg also reported abdominal breathing, ataxia
At the higher doses, decreased activity, piloerection and nasal discharge persisted for 2-3 days.

Mortality was seen in most cases shortly after dosing, preceeded by dyspnea, cyanosis and sometimes convulsions. One female at the highest dose was found dead in the morning the day after dosing.

Summary of mortality

Dose Clevidipine Mg/kg	Mortality ratio	
	males	Females
23	0/2	
33	0/2	
93		1/5
110	0/5	2/5
130	3/5	2/5
140	4/5	5/5
160	2/2	

Median lethal dose estimated at 130 mg/kg.

Summary of body weight changes

Treatment group	Total change days 0-14 (grams)	
	males	Females
Vehicle low vol	49	28
Vehicle high vol	40	20
23 50	48	Not reported
33 72	47	Not reported
93 200	48	22
110 240	48	22
130 280	48	22
140 310	52	-
160 360	-	Not reported

Report 2856/study 93163 Single dose toxicity of clevidipine in mice after IV administration

Clevidipine was given via the tail vein as a single dose over 1 minute. Animals were observed for 14 days after dosing. The dose groups and dosing are summarized below.

Dose groups and dosing

Dose of clevidipine		Dose volume	# and sex
µmol/kg	Mg/kg	Ml/kg	
310	140	1.3	2m
380	180	1.6	2m,5f
410	190	1.7	2m
480	220	2.0	5m,3f
600	270	2.5	5m,5f
720	330	3.0	5m,5f
720	330	3.0	2m
0 vehicle control (dimethyl acetamide)		3.0	5m,5f

Decreased motor activity and respiratory rate were seen in the vehicle control group for up to 4 hours after the dose.

140 mg/kg: both males showed decreased motor activity. 1 showed irregular breathing. Recovery was within 2 hours.

180 mg/kg: splayed gait. 1m died shortly after dosing, first showing dyspnea, cyanosis, And convulsions. Survivors recovered within 5 hours.

190mg/kg: no obvious signs

≥220 mg/kg: survivors showed decreased motor activity, splayed gait, irregular breathing And dyspnea. Signs resolved within 24 hours.

Most deaths occurred shortly after dosing. Three animals in the highest dose groups died approximately 24 hours after dosing and another 3 were euthanized due to deteriorating condition.

Signs of vascular/tissue irritation at the injection site were seen at all dose levels with dose-related increase in severity. Necrosis distal to the injection site was reported for some drug-treated animals.

Dose of H324/38 (µmol/kg)	Excl. sacrificed due to irritation		Mortality ratios	Total	
	M	F		M	F
0 (vehicle control)	0/5	0/5		0/5	0/5
310	0/2	-		0/2	-
380	1/2	0/5		1/2	1/5
410	0/2	-		0/2	-
480	1/5	2/3		2/5	2/3
600	1/5	5/5		4/5	5/5
720[1]	3/5	5/5		3/5	5/5
720[2]	2/2	-		2/2	-

[1]Infusion rate 70 µl/minute
[2]Infusion rate 120 µl/minute

The minimum lethal dose was 180 mg/kg.

2.6.6.3 Repeat-dose toxicity

Report t2966: 5 day intravenous infusion(12 hours per day) dose range-finding study in the rat

Key study findings: A maximally tolerated dose was identified as 137 mg/kg. The target organs of toxicity appear to be the liver, spleen, adrenal and possibly the bone marrow.

Study no.: 265/516
Conducting laboratory and location:
Date of study initiation: April 21, 1994
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: batch # 509/93, purity 98.7%

Sprague-Dawley rats Ico:OFA.SD(IOPS Caw), 8 weeks at start of the treatment. Catheters were implanted at least 2 days before the start of treatment.

Methods

Animals received a daily 12 hour infusion for each of 5 days.

Main study: group A

Dose level		Dose volume	Number of animals	
µmol/kg/day	Mg/kg/day		males	females
50	22	22	3	3
145	66	22	3	3
435	198	66	3	3

Satellite study: group B

Dose level		Dose volume	Number of animals	
µmol/kg/day	Mg/kg/day		males	females
50	22	22	3	3
145	66	22	3	3
435 then 300	198 then 137	66 then 45.6	3	3

Dosing of surviving HD group B animals was stopped on day 2 before the end of the daily treatment period and started 2 days later at 137 mg/kg.

Overall study design

Group number	Group designation	Dose level (µmol/kg/day) Pure test article	(mg/kg/day) Pure test article	Dose volume (ml/kg/day)	Administration rate (ml/kg/hour)	Concentration of H324/38 in formulation (mg/ml)	Number of animals Males	Females
1	Control	0	0	45.6	3.8	0	6	6
2	Vehicle	0	0	45.6	3.8	0	6	6
3	Vehicle	0	0	22	1.84	0	6	6
4	Low dose	50	22	22	1.84	1	6	6
5	Mid dose	145	66	22	1.84	3	6	6
6[2]	High dose	300	137	45.6	3.8	3	6	6

[2] Due to mortality and clinical signs in group 6 satellite B animals, the dose level administered to the main group 6 animals, for which the treatment started 2 days after the satellite study, was reduced to 300 µmol/kg/day.

Group 1 control= saline
Groups 2 and 3 = Intralipid 20%

Morbidity/mortality checks daily. Body weights recorded days 1,3 and 5 with individual food consumption recorded during treatment period.
Blood sampling for determination of test article concentration performed days 1 (Group A, 2 hours after start of infusion) and 5 (Group B satellite group animals, 2 hours after starting infusion) which were then killed without necropsy.

Clinical pathology performed on day 6 for main group animals (euthanized day 6). Selected organs weighed and samples collected for histopathology. Tissues collected

were kidneys, spleen, liver, lungs, heart. Both hematoxylin and eosin and Oil Red O (ORO) were used to stain the sections.

Results

There were 4 occasions when the deviation between target and actual administration rate exceeded 10% for individual animals. This was a total of 4 animals who on one occasion each did not receive the target infusion.

No unscheduled mortality was reported for the main group. One satellite female at the highest dose of 435 μmol/kg was found dead on the 2nd day of treatment after showing subdued behavior.

Treatment related subdued behavior seen in 3 f (300 μmol/kg) between days 1-4. This was also seen in the 435 μmol/kg with greater severity. No other treatment related signs were reported.

Body Weight Gains

While the male groups started at different baseline values, the mean rate of gain was the same between groups. The same was reported for the female groups.

Mean food consumption

Mean food consumption (mean ±SD)

Dose group (mg/kg/day)	Mean food consumption (g/animal/day)	
	males	females
0	32±2.8	22±1.5
0	22±2.6	16±1.4
0	24±4.0	19±2.0
22	25±2.4	18±1.6
66	21±2.9	15±2.0
137	11±5.6	6±1.6

Hematology

Males

There were no consistent changes apparent in the hematology that could be attributed to drug as opposed to vehicle. There was approximately a 30% decrease in platelets at the HD. However, the magnitude of change is within the bounds of inherent variability.

In the differential, an increase in total WBC was seen in all groups apparently due to the vehicle or to the nature of the study. The drug-treated animals showed substantial increases in lymphocytes over the vehicle control groups.

Occasion: Day 6

Gp Sex		WBC k/ mm3 (%) TOTAL	N	E	B	M	L
1M	Mean	12.4	3.8(30)	.1(1)	.0(0)	.0(0)	8.5(70)
	S.D.	2.7	2.2(-)	.1(-)	.0(-)	.0(-)	1.6(-)
2M	Mean	15.9	5.8(34)	.3(2)	.0(0)	.1(1)	9.7(64)
	S.D.	5.7	3.8(-)	.3(-)	.0(-)	.1(-)	2.6(-)
3M	Mean	15.4	5.8(36)	.0(1)	.0(0)	.1(1)	9.4(63)
	S.D.	9.7	4.3(-)	.1(-)	.0(-)	.2(-)	5.6(-)
4M	Mean	16.6	3.9(24)	.3(2)	.0(0)	.0(0)	12.4(74)
	S.D.	6.2	1.5(-)	.2(-)	.0(-)	.1(-)	5.2(-)
5M	Mean	15.7	3.3(21)	.2(1)	.0(0)	.1(0)	12.2(78)
	S.D.	4.7	2.5(-)	.1(-)	.0(-)	.2(-)	3.6(-)
6M	Mean	16.9	4.7(28)	.1(0)	.0(0)	.0(0)	12.1(71)
	S.D.	3.7	1.9(-)	.2(-)	.0(-)	.1(-)	3.3(-)

APPEARS THIS WAY ON ORIGINAL

Females

Drug-treated females showed an increase in reticulocytes. The HD females also showed more of a decrease in platelet counts than the other groups, possibly due to the drug.

Occasion: Day 6

Gp Sex		Hb g/dl	MCH pg	MCHC g/dl	PCV %	RBC M/mm3	MCV fl	RETI /1000	PLAT k/mm3
1F	Mean	13.3	20.5	35.9	36.9	6.45	57	25	1019
	S.D.	2.4	1.2	.8	6.5	1.02	3	11	432
2F	Mean	14.1	20.1	35.7	39.4	7.02	56	17	757
	S.D.	1.2	.7	1.4	2.7	.51	1	7	197
3F	Mean	14.6	20.8	36.0	40.6	7.04	58	23	823
	S.D.	.6	1.4	.6	1.8	.39	4	14	265
4F	Mean	14.5	20.3	35.6	40.7	7.17	57	32	1027
	S.D.	.4	.9	.6	1.5	.38	2	7	67
5F	Mean	13.8	20.4	36.0	38.4	6.79	57	36	788
	S.D.	1.6	.4	.5	4.3	.81	2	16	351
6F	Mean	13.0	19.8	36.1	35.8	6.51	55	30	470
	S.D.	2.6	.8	1.0	6.9	1.18	2	12	420

Females did not show the increase in total WBC seen in the males except for the HD group. Only the HDf showed the increase in lymphocytes seen in the males.

Clinical Chemistry

Males

Serum glucose was increased in the MD and HD males. Total bilirubin was increased above the vehicle effect in the drug-treated males. Creatinine was mildly increased.

Gp Sex		GLUC g/l	BUN g/l	CHOL g/l	TRIGS g/l	FFA mEq/l	T.BIL mg/l	PROT g/l	CREAT mg/l
1M	Mean	.87	.43	.81	.71	1.07	.7	63	4.6
	S.D.	.09	.07	.08	.14	.19	.4	3	.5
2M	Mean	.89	.29	.87	.81	.96	.6	58	4.4
	S.D.	.07	.03	.15	.31	.19	.5	2	.3
3M	Mean	.83	.42	.79	.67	.93	1.1	60	4.5
	S.D.	.08	.08	.15	.06	.26	.5	3	.4
4M	Mean	.87	.42	.70	.61	.83	1.5	58	5.0
	S.D.	.05	.06	.09	.12	.17	1.0	2	.4
5M	Mean	1.04	.38	.80	.63	.95	1.5	60	4.9
	S.D.	.17	.10	.17	.24	.27	.6	1	.4
6M	Mean	1.09	.31	.83	.52	.87	1.9	57	5.1
	S.D.	.12	.04	.16	.13	.21	.5	4	.5

Females

Females showed a mild increase in serum glucose that did not appear to differ from the vehicle. Total bilirubin however, was increased in the drug-treated animals. Creatinine was not appreciably affected.

Gp Sex	GLUC g/l	BUN g/l	CHOL g/l	TRIGS g/l	FFA mEq/l	T.BIL mg/l	PROT g/l	CREAT mg/l
1F Mean	.83	.46	.76	.40	.91	.5	61	5.0
S.D.	.16	.06	.12	.06	.16	.3	3	.3
2F Mean	1.04	.30	.68	.43	.90	.8	59	4.9
S.D.	.14	.02	.21	.07	.26	.4	2	.4
3F Mean	.94	.42	.70	.45	.89	.9	59	5.2
S.D.	.16	.08	.15	.05	.28	.4	4	.4
4F Mean	.89	.38	.72	.46	.96	1.7	59	5.0
S.D.	.12	.03	.08	.06	.26	.5	2	.3
5F Mean	.96	.42	.64	.41	.85	1.9	57	5.4
S.D.	.17	.19	.19	.03	.24	.7	2	.7
6F Mean	1.10	.29	.67	.54	.60	1.9	53	4.9
S.D.	.05	.05	.12	.14	.12	.6	5	.6

Organ Weights

In both males and females, an inconsistent increase in absolute and normalized liver and spleen weights was seen.

When values from the sexes were combined, there was a dose related increase in normalized lung weights. This is a finding consistent with studies where the animals have received an IV infusion.

In this dose ranging study, a maximally tolerated dose was identified as 137 mg/kg. The target organs of toxicity appear to be the liver, lungs, spleen, adrenal and possibly the bone marrow, depending upon the decrease in platelet values. It remains to be seen if these effects are repeated.

Histopathology

From the pathologist's report:

An irritant effect of the drug/vehicle was noted at the injection site, with the highest incidence in the HD group, both sexes considered together.

Treatment related changes were reported for the liver, spleen and lungs. The primary change in the liver was single cell necrosis and increased mitotic activity. This was seen in all groups, including control, with no dose-related incidence
Sinusoidal histiocytosis was also seen across all groups with no dose related incidence.

> Most animals showing histiocytic infiltration in the liver also had an increase of histiocytes in the spleen. As in the liver, these histiocytes did not stain positively with ORO.
> The ORO staining in other macrophages in the spleen was, however, slightly increased in lipid control groups (2 and 3) and in treated groups (4 to 6) compared with the controls, however, there were no intergroup differences in severity.
>
> In the lungs of male 25 (group 5), moderate and multifocal haemorrhages were seen associated with dilatation of alveolar capillaries.
> Positive ORO staining was seen in the lumen of pulmonary vessels from animals given the vehicle control (lipid) and treated animals with a higher severity in males and females from groups 5 and 6 (especially in the group 5 male n° 25 which showed dilatation of the capillaries).

The histiocytosis was given partial attribution for the increase in liver weights due to poor correlation. Changes in spleen weight were also considered to be related to the histiocytosis. The histiocytosis was thought to be due to the lipid vehicle.

> The apparent dose related increase in adrenal weight in females treated with the test article when compared to the saline controls indicated a possible effect of the test article. Such changes could also possibly be related to stress or a combination effect. However, as this change was not present in males, it is unlikely that it was directly related to stress (adrenals were not examined histologically).

This statement becomes more interesting in light of the following paragraph:

The positive ORO staining in the lumen of the vessels of the lungs in all treated groups (including lipid controls) could be due to the presence of small lipid emboli. This occured at a higher incidence in males and females from groups 5 and 6 when compared with others receiving lipids.
The apparent increases in lungs weights were due to incidental changes in some animals of these groups (e.g animal N°25 in group 5 with haemorrhages, animal N°23 with bronchopneumonia in group 4, animal N°71 in group 6 with interstitial pneumonia).

Pulmonary emboli are typically seen secondary to adrenal malfunction, something that would not have had time to manifest clinically in this short duration study. This is worth looking out for in the longer term studies. Another question is whether the emboli are secondary to inadequate maintenance of the catheters.

Study title: ***Complementary Study: 7 day continuous IV (12 hours per day) infusion study in the rat.***

Key study findings: No findings of toxicologic significance. Part of the purpose of this study was to see if the ocular lesions from an earlier study repeated. They did not.

Study no.: report t2969/study 265/523
Conducting laboratory and location:
Date of study initiation: November 18, 1994
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: batch # 111/94

Methods

Sprague Dawley rats (Ico:OFA.SD) were used.
The sponsor's summary of the study design is shown below.

Group number	Dose level (µmol/kg/day)	(mg/kg/day) Pure test article	Administration rate (ml/kg/hour)	Concentration of H324/38 in formulation (mg/ml)	Number of animals									
					Males					Females				
					Sets	A.	B.	C.	D	Sets	A.	B.	C.	D
1	0	0	1.84	0	4,	0,	0,	4		4,	0,	0,	4	
2	0	0	1.84	0	4,	0,	0,	4		4,	0,	0,	4	
3	14	6.6	1.84	0.3	0,	4,	4,	4		0,	4,	4,	4	
4	48	22	1.84	1	0,	4,	4,	4		0,	4,	4,	4	
5	145	66	1.84	3	0,	4,	4,	4		0,	4,	4,	4	

Sets A, B and C were included for blood sampling for determination of test article blood concentration. Set D was included for ophthalmological examinations.

Group 1 received saline. Group 2 received the vehicle, Intralipid 20%.
Three sets of animals were used: A, B,C.

A: sampled days 1 and 7, once a day
B: sampled days 4 times on day 1
C: sampled 4 times on day 7

Times of sampling: set A: 6 hours after start of infusion
Set B and C: before start of infusion
2,6 and 12 hours after start of infusion

Animals were examined for signs, body weight, and ophthalmic changes. At euthanasia, the animals were subject to gross necropsy. Only the eyes and optic nerves were collected. No histopathology was performed.

Results

No treatment related signs were reported. One group C male was found dead before the 2 hour blood sampling. It was assumed that the death was anesthesia-related due to lack of macroscopic findings to explain the death.

The pathologist's report states that the aim of the study was to confirm the test article blood levels and the ocular abnormalities in a previous 4 week infusion study in the rat. No treatment related changes were reported at necropsy. Numerous ophthalmic findings were recorded, but the sponsor did not consider any to be drug-related vs procedure related.

Study title: ***4 week intravenous infusion(12 hours per day) toxicity study in the rat***

Key study findings: The fact that almost every animal on this study was ill with systemic sepsis makes this a study of no particular value.

Study no.: report t2968/study 265/520
Conducting laboratory and location: [redacted]
Date of study initiation: August 25, 1994
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: batch # 111/94, purity 100%

Methods Sponsor's summary is shown below.

MAIN GROUPS

Group number	Group designation	Dose level (µmol/kg/day) Pure test article	Dose level (mg/kg/day) Pure test article	Dose volume (ml/kg/day)	Administration rate (ml/kg/hour)	Concentration of H324/38 in formulation (mg/ml)	Number of animals Males	Number of animals Females
1	Control	0	0	22	1.84	0	10	10
2	Vehicle	0	0	22	1.84	0	10	10
3	Low dose	14	6.6	22	1.84	0.3	10	10
4	Mid dose	48	22	22	1.84	1	10	10
5	High dose	145	66	22	1.84	3	10	10

SATELLITE GROUPS : SETS A, B and C

Group number	Group designation	Dose level (µmol/kg/day) Pure test article	Dose level (mg/kg/day) Pure test article	Dose volume (ml/kg/day)	Administration rate (ml/kg/hour)	Concentration of H324/38 in formulation (mg/ml)	Number of animals Males Set A,B,C,	Number of animals Females Set A,B,C,
3	Low dose	14	6.6	22	1.84	0.3	4, 4, 4	4, 4, 4
4	Mid dose	48	22	22	1.84	1	4, 4, 4	4, 4, 4
5	High dose	145	66	22	1.84	3	4, 4, 4	4, 4, 4

SATELLITE GROUPS : SET D

Group number	Group designation	Dose level (µmol/kg/day) Pure test article	Dose level (mg/kg/day) Pure test article	Dose volume (ml/kg/day)	Administration rate (ml/kg/hour)	Concentration of H324/38 in formulation (mg/ml)	Number of animals Males	Number of animals Females
1	Control	0	0	22	1.84	0	4	4
2	Vehicle	0	0	22	1.84	0	4	4

Group 1 animals (control) received the control article (physiological saline).
Group 2 animals received the vehicle (Intralipid® 20 % for infusion).

Observations included morbidity/mortality checks, signs, body weights and food consumption, ophthalmological exams(pre-test and week4).
Blood sampling for test article determination days 1,7 and 28 for satellite animals.
Urine was collected in metabolism cages from animals given a bolus of water by gavage prior to start of collection then deprived of water during the collection period.
Clinical pathology on day 29 (necropsy) for main group animals.

- Animals examined and frequency : four different sets of satellite animals were used : sets A, B, C and D (see § 4.2.).
  - Set A : animals were sampled 4 times on day 1,
  - set B : animals were sampled 4 times on day 7,
  - set C : animals were sampled 4 times on day 28,
  - set D : animals were sampled on days 1, 7 and 28, once a day.

- Time of sampling :
  - sets A, B and C, on day 1 or 7 or 28 :
    - before start of the infusion pump,
    - 2, 6 and 12 hours after the start of the infusion pump ;
  - set D : on days 1, 7 and 28, 6 hours after the start of the infusion pump.

Animals were subject to gross necropsy. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroids. Histopathology was performed on a standard list of tissues including bone marrow from the sternum.

Results

Deviation between actual and theoretical administration rate for each infusion pump was generally not more than 10% except on a few occasions generally due to technical problems. From day 22, a white precipitate was noted in the catheter of most HD animals and a very few of group 3 and 4 males. The sponsor notes that this might explain the higher number of catheter reimplantations in group 5 than in other groups.

Mortality

Dose(mg/kg/day)	Day of death	males	Females
0			
0		1	1
6.6		1	
22		1	1
66		8	4

All premature mortalities were reported to show similar clinical signs prior to death. These included piloerection, red coloration of the eyelids or around the muzzle, subdued behavior, cold to the touch, difficult respiration and/or distension of the abdomen.

Ophthalmic findings

As in the 7 day study, corneal opacities were seen in all dose groups at week 4. It was reportedly not dose related and also seen in controls. Corneal neovascularizations were reported only for the drug-treated groups and associated with "corneal hemorrhage". This was described as possibly related to the test article.

Sepsis

Something not mentioned until page 25 was the incidence of systemic sepsis. The sponsor's incidence table is shown below.

	Decedent rats	All rats on study
Saline control	0	7/20
Vehicle control	2/2	15/20
Low dose	1/1	10/20
Intermediate dose	2/2	20/20
High dose	11/12	18/20

The fact that almost every animal on this study was ill with systemic sepsis makes this a study of no particular value.

Study title: ***One-month continuous IV infusion toxicity study in the rat***

Key study findings: The interpretation of this study is confounded by the very poor survival. Approximately 10% or 2/20 animals per group survived to scheduled euthanasia. There is some inconsistency in the numbers of samples collected for analysis of certain parameters.

Study no.: Report 96008/ Study
Conducting laboratory and location: Astra Charnwood, Leicestershire, UK
Date of study initiation: July 10, 1996
GLP compliance: statement p. 46
QA report: yes (x) no ( )
Drug, lot #, and % purity: AH-005(~97%), HL-003(97%), AS-007(~97%)

Methods

Three groups of 10 male and 10 female Sprague-Dawley rats were dosed with clevidipine for a minimum of 28 days at doses of 23, 39 and 66 mg/kg/day. Further groups of 10 males and 10 females received either saline or Intralipid 20%. Additional groups of 10m and 10f received either saline or clevidipine for one month and then observed for a drug-free recovery month.

Animals were observed for signs, bodyweights, food and water consumption, blood sampling for hematology and clinical chemistry, urinalysis after 4 weeks dosing. Ophthalmoscopic exams were conducted pre-test and prior to the end of the study. At necropsy, a standard list of tissues was collected for histological examination.

Results

Clinical signs were reported for all groups and were most likely procedure-related. Signs included: soiling of the body, ungroomed appearance and "reddened" eyes.

As shown in the sponsor's summary table, food consumption was decreased in all groups except for the saline control. Body weight was not affected, possibly due to the caloric content of the Intralipid vehicle.

The sponsor provides the following summary tables :

Dose Group	1	2	3	4	5	6	7
Dose level ($\mu mol \cdot kg^{-1} \cdot day^{-1}$)	0 (saline control)	0 (intralipid)	50	85	145	0 (saline recovery)	145 (recovery)
No. of Animals/sex	20	10	10	10	20	20	20
This study was carried out in compliance with GLP							
NOTEWORTHY FINDINGS							
GROUP SURVIVAL							
Dose Group	1	2	3	4	5	6	7
Males	18/20	8/10	8/10	10/10	2/20	20/20	0/20
Females	9/10	10/10	9/10	10/10	9/10	10/10	6/10
FOOD CONSUMPTION ($g \cdot animal^{-1} \cdot day^{-1}$) Mean (± SD)							
Males - Week 1	34.1 (± 5.6)	29.9* (± 3.1)	29.6** (±2.9)	30.5* (±1.6)	26.8*** (±3.6)	N/A	N/A
Females - Week 1	27.0 (± 2.8)	24.5* (±2.4)	24.0* (±2.7)	23.5** (±1.9)	22.3*** (±1.7)	25.8 (±1.9)	21.4*** (±3.6)
WATER CONSUMPTION ($ml \cdot animal^{-1} \cdot day^{-1}$) Mean (± SD)							
Males - Week 3	32.7 (±7.5)	32.1 (±9.6)	37.4 (±4.8)	44.5** (±11.5)	N/A	N/A	N/A
Females - Week 3	34.3 (±8.7)	30.1 (±13.1)	32.1 (±6.8)	37.8 (±7.4)	42.9* (±8.5)	27.7 (±5.0)	41.8 (±9.6)

* p<0.05
** p<0.01
*** p<0.001

Water consumption was significantly increased in the drug-treated animals compared to the vehicle controls.

Keratitis, or corneal inflammation, was reported for all groups. Both unilateral and bilateral keratitis was reported. This is a profoundly painful condition and may possibly influence hematology/clinical chemistry parameters. More than that, keratitis has been

listed as occurring in a very high level in almost every rodent study associated with this NDA. This argues that either the animal husbandry or the procedures are at fault. It is entirely possible that during the surgical implantation of the catheters, the corneas were not protected with a sterile ophthalmic lubricant. This step was not mentioned in the Methods Section. The lack of attention to this basic humane detail is disturbing.

Table 6: Summary of Ophthalmoscopy - Incidence of Keratitis

Group				
1	2	3	4	5 and 7*
4/30 (13%)	3/20 (15%)	3/20 (15%)	3/20 (15%)	6/34 (18%)

* High doses combined.

NOTEWORTHY FINDINGS (Continued)							
CLINICAL CHEMISTRY AT TERM - URINE							
Volume (ml) - males	6.2 (±3.6)	3.3* (±1.5)	6.6 (±2.3)	9.6 (±5.6)	10.8 (± 0)	N/A	N/A
Volume (ml)- females	6.6 (±3.3)	5.2 (±2.8)	6.4 (±2.5)	10.7 (±8.1)	10.9* (±4.4)	N/A	N/A
MEAN ORGAN WEIGHTS (mg)							
Adrenals - Males	77.7 (±11.5)	80.1 (±13.3)	78.1 (±8.6)	91.0* (±13.9)	N/A	N/A	N/A
Ovaries - Females	98.0 (±14.2)	97.7 (±12.6)	108.7 (±12.8)	114.6* (±19.0)	119.1* (±23.7)	108.6 (±14.5)	99.1 (±21.0)

TOXICOKINETICS - DAY 7							
Dose Group	1	2	3	4	5	6	7
Clevidipine - Males (Range - nmol·l^{-1})	N/A	-	9.3 - 52.6	19.7 - 84.7	21.3 - 170	N/A	N/A
Clevidipine - Females (range - nmol·l^{-1})	N/A	-	<5.0- 2710	5.5 - 41.3	8.5 - 206	N/A	N/A
H 152/81 - Males (range - µmol·l^{-1})	N/A	-	75.3 - 136	134 - 152	124 - 204	N/A	N/A
H 152/81 - Females (range - µmol·l^{-1})	N/A	-	98.4 - 142	126 - 235	242 - 280	N/A	N/A
There were no histopathological findings that were attributable to treatment with clevidipine. Effects due to Intralipid® were principally related to the liver.							

* $p<0.05$
** $p<0.01$
*** $p<0.001$

Body weights

Drug-treated and lipid vehicle animals gained more weight on average than the saline control group.

Mean weights (g) for males

Dose group µmol/kg/day	Day 1	Day 29	Mean difference Day1 to day 29
0	382	468	86
0	376	472	96
50	381	479	98
85	390	483	93
145	383		

Mean food consumption was decreased in groups 2-5 but was apparently compensated for by the caloric content of the Intralipid.

Mean weights (g) for females

Dose group	Day 1	Day 29	Mean difference Day1 to day 29	Day 57	Mean difference Day 57 to day 29
0	260	289	29		
0	260	289	29		
50	262	300	38		
85	261	294	33		
145	260	289	29		
Saline recov	251	292	41	302	10
145 recov	248	293	45	298	5

Mean food consumption was decreased in groups 2-7 through the 4 weeks of drug treatment but was apparently compensated for by the caloric content of the Intralipid. During the recovery period there was no difference in food consumption between vehicle and drug-treated females.

Water consumption was increased in both sexes as summarized in the sponsor's tables.

Table 10: Group Mean Water Consumption (ml·rat^{-1}·day^{-1}) - Males

		Week			
		1	2	3	4
Dose Group					
1	Mean	28.24	30.95	32.73	32.54
	SD	4.79	5.23	7.46	7. 67
	N	10	10	8	8
2	Mean	28.21	29.50	32.11	26.78
	SD	5.06	4.90	9.60	9.31
	N	10	10	10	9
3	Mean	34.76*	34.81	37.35	37.63
	SD	9.84	6.17	4.76	5.44
	N	10	10	10	9
4	Mean	36.91**	42.23***	44.54**	42.98**
	SD	5.04	8.00	11.47	8.79
	N	10	10	10	10
5	Mean	42.17***	46.35***		
	SD	7.41	9.75		
	N	10	10		

For comparisons with Group 1

* p<0.05
** p<0.01
*** p<0.001

Table 10 (Continued): Group Mean Water Consumption (ml·rat^{-1}·day^{-1}) - Females

		Week							
		1	2	3	4	5	6	7	8
Dose Group									
1	Mean	28.09	32.20	34.27	32.08				
	SD	5.93	8.04	8.74	4.80				
	N	10	10	9	9				
2	Mean	24.92	27.41	30.05	21.92*				
	SD	7.21	12.79	13.10	6.70				
	N	9	9	9	7				
3	Mean	28.59	29.19	32.14	31.51				
	SD	5.03	7.80	6.80	6.16				
	N	10	10	9	9				
4	Mean	32.59	33.23	37.80	36.07				
	SD	5.48	5.38	7.35	7.32				
	N	10	10	10	10				
5	Mean	32.70	37.79	42.94*	37.54				
	SD	3.08	5.18	8.45	10.84				
	N	10	10	10	9				
6#	Mean	22.46	26.64	27.67	28.23	30.44	30.07	30.37	30.83
	SD	4.44	6.93	4.95	7.55	5.08	7.80	8.37	8.19
	N	10	10	10	10	10	10	10	10
7	Mean	30.13	36.32	41.77	41.23*	30.18	28.10	31.64	31.83
	SD	4.24	7.55	9.61	8.18	4.35	4.71	6.42	6.73
	N	10	10	8	8	7	6	6	6

For comparisons with Group 1: * p<0.05; ** p<0.01; *** p<0.001

Data not included in the statistical analysis.

There were no effects of toxicological significance in the clinical chemistry as reported.

Hematology

There was a decrease in platelet count up to 20% associated with vehicle. Hemoglobin, rbc, HCT and MCV were decreased in the HD females while reticulocytes were increased (regenerative anemia). There were no significant differences in the recovery group animals.

Something not mentioned in the summary table is the effect on testes, liver or thyroid weight. Because the HD group was not evaluated, it is difficult to say if there was a trend or if normal variability accounted for the apparent changes.

SEX: MALES		GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7
		0.92 ml/kg/h	0.92 ml/kg/h	50 um/kg/d	85 um/kg/d	145 um/kg/d	0.92 ml/kg/h	145 um/kg/d
TESTES	MEAN	3.525	3.519	3.288*	3.144		2.400**	
	S.D.	0.244	0.229	0.158	0.741		0.735	
		(8)	(8)	(8)	(10)		(2)	
PROST-ATE	MEAN	0.665	0.552	0.653	0.657		0.792	
	S.D.	0.158	0.192	0.212	0.255		0.260	
		(8)	(8)	(8)	(10)		(2)	
LIVER	MEAN	16.31	16.25	15.83	17.06		20.18	
	S.D.	2.70	2.08	1.59	1.92		3.77	
		(8)	(8)	(8)	(10)		(2)	
LEFT THYROID	MEAN	16.2	14.2	14.3	15.6		21.1	
	S.D.	4.8	2.2	3.6	3.1		11.7	
		(8)	(8)	(8)	(10)		(2)	

Normalized (relative to body weight) testes weight was also affected, showing a dose-related decrease. Reversibility was not assessed.

TESTES	MEAN	0.759	0.747	0.690	0.660		0.467**
	S.D.	0.069	0.041	0.064	0.178		0.143
		(8)	(8)	(8)	(10)		(2)

Abnormalities (oligospermia and round spermatids) were reported only for the Intralipid vehicle control group. However, given the number of animals who did not complete the study, this may not be the total picture.

Normalized adrenal weight was unaffected in males.

Liver and adrenal weight was also affected in the females. This appears to be due to the vehicle.

SEX: FEMALES		GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7
		0.92 ml/kg/h	0.92 ml/kg/h	50 um/kg/d	85 um/kg/d	145 um/kg/d	0.92 ml/kg/h	145 um/kg/d
ADRENAL (BOTH)	MEAN	76.756	108.220*	86.089	90.010*	109.978**	78.470	74.983
	S.D.	10.029	39.456	19.969	12.352	19.472	20.044	5.589
		(9)	(10)	(9)	(10)	(9)	(10)	(6)

LIVER	MEAN S.D.	11.24 1.20 (9)	13.25** 1.57 (10)	12.04 0.89 (9)	12.54 1.63 (10)	14.89** 2.48 (9)	11.45 1.44 (10)	12.13 1.28 (6)

Normalized (relative to body weight) adrenal and ovary weight was increased while normalized kidney weight was decreased.

Histopathology

	males				females			
Group#	1	2	5	6	1	2	5	6
Animals on study	20	10	22	20	10	10	11	10
Animals completed	2	2	20	0	1	0	2	0
Myocardial degeneration/fibrosis	0	1	1	0	0	0	0	0
Myocardial inflammation	0	1	5	0	0	0	0	0

This is appalling.

The majority of HD males were removed from the study between weeks 3 and 4 due to blocked cannulae. This was initially believed to have resulted from filtration of the high dose formulation through a 5 μM filter prior to dosing. The sponsor believed that the filtration had destabilized the emulsion. The procedure was halted prior to dosing the females with a reported improvement in catheter patency. When repeated though, the male HD cannulae again became blocked. This time the precipitation was believed to be due to longer storage time of the formulation. No stability data?

Study title: Intralipid: One month continuous intravenous infusion investigative toxicity study in the rat.

Key study findings: Poor survival and lack of untreated or saline control groups somewhat confounds the interpretation. There was little detectable effect except that the rats didn't survive 1.27ml/kg/hour Intralipid. The sponsor summarized that — 20% Intralipid infused at 0.92ml/kg/hr was acceptable over a one month period.

Study no.: Report SE10192-1/study SE10192
Conducting laboratory and location: Astra Charnwood, Leicestershire, UK
Date of study initiation: March 28, 1996
GLP compliance: statement located
QA report: yes ( ) no (x)

Drug, lot #, and % purity: Intralipid, batches 73172-51, 81724-51

Methods

The study was to investigate the suitability of Intralipid for intravenous infusion in the rat. Three groups of 3 female Sprague Dawley rats were dosed with Intralipid. 20% w/v, for at least 28 days at either 0.92 or 1.27 ml/kg/hr. A single male receiving 20% Intralipid at 0.92 ml/kg/hr was also examined.

Animals were examined for signs, bodyweights, clinical chemistry, hematology and limited necropsy (samples of liver, lungs, kidneys and mesenteric lymph nodes).

Results

Two females treated with 20% Intralipid at 1.27 ml/kg/hr were euthanized due to poor condition. The male infused with 20% Intralipid was also euthanized due to poor condition but for some reason this was considered unrelated to treatment. The high infusion rate females showed signs of staining around the eyes, nostrils, urogenital region, "noisy respiration", dyspnea, distended abdomen and hunched posture. The male rat showed signs of "noisy respiration", hunched posture, staining as was seen in the females, lethargy and hematuria.

One out of 3 female rats infused with at 0.92 ml/kg/h showed signs (staining and weakness in the hindlimbs).

Table 2: Summary of Clinical Observations

Dose Group	Number of Animals	Number of Animals Affected on One or More Occasions					
		Blood in Urine/Cage	Distended Abdomen	Staining: Facial and/or Urogenital	Noisy Respiration	Irregular Respiration	Abnormal gait, hunched
1	1 Male	1/1	1/1	1/1	1/1	1/1	0/1
1	3 Females	1/3	0/3	1/3	0/3	0/3	0/3
2	3 Females	0/3	0/3	1/3	0/3	0/3	0/3
3	3 Females	0/3	2/3	2/3	1/3	0/3	1/3