Bodyweights

Given the small sample size and the limited number of animals surviving, very little can be said regarding trends in weight gain.

Summary of body weights (g)

Animal#	Dose group	Day1	Day 29	Difference in weight (g)
1	1	448	Died D22	----
11	20% intralipid	238	267	29
12		213	291	78
13		247	276	29
21	2	227	287	60
22	[redacted]Intralipid	237	252	15
23		188	220	32
31	3	263	Died	----
32	20%Intralipid	222	Died	----
33		223	Died D22	----

Clinical Chemistry

There is no untreated group for comparison and no historical control values provided. Group 2 females showed a mean increase in blood urea. One female in this group also showed an increase in serum creatinine and phosphorous. The same animal also showed a marked increase in ALP (approx 3X compared to the other animals).

Hematology

The same difficulties apply to the hematology: a limited number of animals and no basis for comparisons.

		females		
	Male rat	Group1	Group 2	Group 3
pyelonephritis	0	0	1	0
Hepatic periportal vacuolation	0	1	2	0
Hepatic inflammatory cell foci	0	0	1	0
Hepatic periportal fat deposition	0	2	2	0

The sponsor concluded that clinical chemistry revealed plasma changes consistent with infusion of a lipid rich material, including raised levels of urea and creatinine, increased γ-glutamyl transferase, total bilrubin, alkaline phosphatase, aspartate aminotransferase and lowered albumin.

The sponsor also concluded that the pathology reflected effects on the liver and kidney. Pyelonephritis (reported in 1 animal) was observed, and it was hypothesized that this was associated with infection disseminated from the infusion site. Minimal hepatocyte vacuolation associated with fat deposition was also assumed to be associated with lipid infusion. The histopathology summary was limited, primarily focusing on liver, lung, kidney and lymph node.

I found the sponsor's final statements very interesting:

> Rats treated with 20% Intralipid® at 1.27 ml·kg^{-1}·h^{-1} showed little differences pathologically from the other groups, but this infusion rate was clearly shown to be unsustainable over the planned infusion period.

In other words, there was little detectable effect except that the rats didn't survive. The sponsor summarized that — 20% Intralipid infused at 0.92ml/kg/hr was acceptable over a one month period.

Study title: ***5 day intravenous infusion (12 hours per day) dose range-finding study in the Beagle dog***

Key study findings: There were no findings of toxicological significance in this study. Dosing seems to be limited by the secondary effect of sedation.
Report Number: T2956
Study no.: 265/517
Conducting laboratory and location: [redacted]
Date of study initiation: April28, 1994
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: H324/38, batch 509/93, 98.7%

Methods

The sponsor summarized the design of the study in the following table:

Group number	Group designation	Dose level		Dose volume	Dose concentration	Infusion rate	Number of animals	
		µmol/kg/day	mg/kg/day	ml/kg/day	mg/ml	ml/kg/hour	Males	Females
1	Control	0	0	32.8	0	2.73	2	2
2	Vehicle	0	0	32.8	0	2.73	2	2
3	Low dose	35	16	5.3	3	0.44	2	2
4	Intermediate dose	70	32	10.6	3	0.88	2	2
5	High dose	215	98	32.8	3	2.73	2	2

Prior to the start of the study, silastic catheters were implanted into a jugular vein of each animal. "Control" animals received physiologic saline: and the vehicle group received Intralipid 20%. Drug was administered daily for 12 hours of continuous infusion.

On day 5, an error in programming of the infusion pump was made for group 3 animals. These animals were treated at half of the theoretical administration rate. These animals were treated again on day 6 for approximately 2 hours to repeat the blood sampling for plasma level determination of the test article.

Morbidity/mortality checks were performed twice daily. Body weights were recorded three times weekly and food consumption was measured daily.

Blood sampling for the test article concentration was performed on days 1 and 5 for group 3-5 animals and on day 6 for group 3 animals only.

Clinical pathology (hematology and a limited clinical chemistry panel) was examined twice pretest and on day 6. All animals were euthanized and necropsied Day 6. Selected organs were weighed and tissue samples preserved. Selected tissues were examined histopathologically. The tissue sampling was not comprehensive and included: adrenals, bone marrow smears, brain, heart, injection site, kidneys, liver, lungs, esophagus, ovaries, pancreas, prostate, spleen, stomach, testes, thymus, thyroids, trachea, gross lesions.

The cardiovascular system was examined day 1 and 5 (day 6 for the group 3 animals)before the start of infusion and 1 and 2 hours after the end of infusion. Lead I, II, and III ECGs were obtained from conscious animals. The ECGs were analyzed for HR, rhythm, ST segment and QRS, PR and QT intervals.

Results

The achieved dose fell within the target of ±10% of the projected dose.

No unscheduled mortality was recorded.

≥70 µmol/kg/day : subdued behavior and prolapsed nictitating membranes in 2/2MDf, 2/2 HDm, 2/2HDf

Peripheral vasodilation: "most" of the animals treated with the test substance.

Body weight

Only the males receiving the saline infusion showed any weight loss. All others gained some weight with no discernible drug-related effects.

Reviewer's summary of mean body weight changes (kg) for males

group	Day -7	Day 5	Change day -7 – day5
saline	7.7	7.6	-1
Intralipid	7.9	8.3	0.4
35µmol/kg/day	9.4	9.7	0.3
70µmol/kg/day	8.3	8.8	0.5
215µmol/kg/day	8.2	8.5	0.3

Reviewer's summary of mean body weight changes (kg) for females

group	Day -7	Day 5	Change day -7 – day5
saline	6.2	6.5	0.3
Intralipid	7.6	8.0	0.4
35µmol/kg/day	8.9	9.1	0.2
70µmol/kg/day	7.6	8.1	0.5
215µmol/kg/day	6.7	6.9	0.2

Tachycardia was seen day 1 (1 and 2 hours) and on day 5 (1 and 2 hours) in the drug treated groups of both sexes. This is consistent with a reflex effect due to blood pressure lowering. The effect was also present day 6 for the animals of group 3.

PR interval in males was not appreciably affected in males or females. There were no appreciable effects in any of the measured parameters. QT correction was not done.

Hematology

There was some variability in the pre-test hematology which suggests that the post-test values should be interpreted cautiously. The decrease in PCV may be a real phenomenon although it was not manifest to the same degree in both sexes. The sponsor felt that there might also be an increase in serum cholesterol, but it appears to be within the level of variability and stress.

Pathologist's Report

A treatment related change was reported for the liver. Hepatocytes with clear cytoplasm were seen in the periportal areas. In some areas the cells appeared to have the rarefied cytoplasm associated with glycogen deposition and in other areas had the vacuolated appearance usually associated with fat. The cells did not stain positively with Oil Red O. The incidences were: 0(saline), 1f(Intralipid), , 2MDf, 1HDm, 1HDf.

Study title: ***Vaso- and tissue irritation study in dogs of H324/38 given intravenously and subcutaneously for 5 days***

Key study findings: There was some swelling associated with subcutaneous injection of vehicle. There was no increase in irritative potential with increased dose, indicating that the vehicle was the irritant.
Report: t2950
Study no.: 824-03
Conducting laboratory and location: Astra Safety Assessment, Sweden
Date of study initiation: September 8, 1994
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: H324/38 batch 111/94, purity 99.6%

Methods

This study examined the irritative properties of clevidipine given to dogs for 5 days. The test compound was dissolved in a commercial fat emulsion (Intralipid®).

Each group consisted of 1 male and 1 female dog. IV doses were given as 2 hour infusions. The subcutaneous dose was given 2 hours after the IV infusion. The animals were necropsied 3 days after the final dose.

		INTRAVENOUS				
Group	Compound	Daily dose nmol/kg/min	µg/kg/min	Inf. volume ml/kg	Inf. rate ml/kg/h	Inf. time min.
1	Control	0	0	1.09	0.55	120
2	Vehicle	0	0	1.09	0.55	120
3	H324/38	10	4.6	0.55	0.28	120
4	H324/38	20	9.1	1.09	0.55	120
5	H324/38	60	27	1.09	0.55	120

		SUBCUTANEOUS			
Group	Compound	Daily dose µmol/dog	mg/dog	Volume ml/dog	Formulation
1	Control	0	0	1.0	I
2	Vehicle	0	0	1.0	II
3	H324/38	2.2	1.0	1.0	III
4	H324/38	-	-	-	III
5	H324/38	6.6	3.0	1.0	IV

The IV doses were administered via the cephalic vein of the right leg. The subQ doses were given in the left leg, alongside the cephalic vein.

Results

Four dogs showed swelling at the subQ injection site:
2 on day 4 (1 mg/ml)
2 on days 1, 2,3 and 4 (3mg/ml)
A slight swelling was also reported for one of the dogs receiving subQ saline.

One control dog had a minimal fibrinous thrombus adhering to a vessel venous valve leaflet.

1 group-4 dog (20 µmol/kg.min) had a small fibrinous thrombus adherin to ~1/5th of the vessel wall circumference. Minimal thrombophlebotic signs were reported in the vessel wall. The sponsor qualifies this by reporting paravenous hemorrhage and speculating that the signs were due to the injection cannula.

No signs of thromboembolism were reported for the lungs. One female dog in the MD group and one in the HD group had bronchopneumonia that was proposed to precede the treatment. Deposition of fat from the vehicle was seen to some extent in all dogs at the subcutaneous injection site, including the dogs receiving the vehicle.

Lacking an increase in reported signs of irritation with increased dose, it would seem reasonable to assume an irritative potential in the vehicle.

Study title: ***Primary skin irritation study in the rabbit***

Key study findings: Within 72 hours of dermal application of the test article there were no observed signs of irritation.

Study no.: T3107
Conducting laboratory and location: [redacted]
Date of study initiation: October 23, 1995
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: H324/38, batch 300/94 purity ~99%

Methods

Three female NZW rabbits were exposed to test article at two clipped skin sites on the back. Test article (0.5 g) moistened with 0.5 ml distilled water was applied to each of 6 gauze patches. Gauze patches were secured with tape over the skin sites. After 4 hours of exposure the test article was removed and the skin was examined 1, 24, 48 and 72 hours after termination of exposure.

Erythema , eschar and edema were scored on a scale of 0-4 with 0 being "not present" and 4 being the most severe changes.

No abnormalities were reported for any of the assessed parameters. The mean individual erythema scores were 0.0 for all 3 rabbits. The mean individual edema scores were 0.0 for all 3 rabbits. The mean score for the test article was 0.0 erythema and 0.0 for edema.

The sponsor concluded that the drug was to be classified as non-irritating.

2.6.6.4 Genetic toxicology

Study title: Bacterial Mutation Test

Key findings: Substantial increases in revertant colony counts were obtained with strains TA98, TA100 and TA102 following exposure to clevidipine in the presence of the S9 mix. Substantial increases in revertant colony count were also obtained with the same strains following exposure to the positive control, formaldehyde (except with the strain TA102) in the presence of S9 mix. These results were repeated in the confirmatory assay. Formaldehyde dehydrogenase added to the test system decreased but did not totally eradicate the increase in revertants.

Study no.: report961056
Conducting laboratory and location:

Date of study initiation: April 18, 2006
GLP compliance: statement included
QA reports: yes (x) no ( )
Drug, lot #, and % purity: clevidipine, lot 2930.D.03.5, dissolved in DMSO. Purity reported as 100% by HPLC
Formaldehyde, 37% was used for comparison
Positive controls in absence of S9: sodium azide (NaAz), 9-aminoacridine (AC) , 2-nitrofluorene (2NF), mitomycin C (MMC), 4-nitroquinoline N-oxide(NQO)
Positive controls in presence of S9: 2-aminoanthracene(2AA), benzo[a]pyrene (BaP)

Methods

Bacterial strains used: S.typhimurium TA1535, TA1537, TA98, TA100, TA102 and E.coli WP2 *trp uvr A.*
Commercially available rat S9 mix was used.
Formaldehyde dehydrogenase (FDH) from Pseudomonas putida was prepared to a final activity of 4 units/ml for use with TA98 and 40 units per ml for use with TA102 and TA100. The sponsor states that these concentrations were optimized for detoxification of formaldehyde based on experiments not reported here.

The initial test and the confirmatory test were both by the pre-incubation method.
Initial test study design

material	Formulation conc (µg/ml)	Conc µg/plate	Number of replicates -S9	Number of replicates +S9	# of strains
Vehicle	-	-	3	3	6
Test article	562	28.1	3	3	6
	1000	50	3	3	6
	1778	89	3	3	6
	3162	158	3	3	6
	5623	281	3	3	6
	10000	500	3	3	6
	17783	889	3	3	6
	31623	1581	3	3	6
	56234	2812	3	3	6
	100000	5000	3	3	6
Reference article	**	**	3	3	3[a]
Positive control	‡	‡	3	3	6

‡depends on test organism and positive control agent

Confirmatory test design

material	Formulation Conc (µg/ml)	Conc (µg/plate)	Strains tested			
			-S9	+S9		
			No FDH	No FDH	0.1UFDH[b]	1UFDH[c]
Vehicle	-	-	all	all	TA98[d]	TA100,TA102
Test article	562	28.1	all	all	TA98	TA100,TA102
	1000	50	all	all	TA98	TA100,TA102
	1778	89	all	all	TA98	TA100,TA102
	3162	158	all	all	TA98	TA100,TA102
	5623	281	all	all	TA98	TA100,TA102
	10000	500	all	all		TA102
	17783	889	all	all		TA102
	31623	1581	all	all		TA102
	56234	2812	all	all		-
	100000	5000	all	all		-
Ref. article	**	**	all	all	TA98	TA100,TA102
+control	‡	‡	all	all	-	-

‡ Depends on the test organism and the positive control agent used
** Refer to section 8.4
† All = all six strains, 0.1 and 1U FDH indicates units of FDH per plate
¶ 3 replicate plates were tested at each experimental point in the confirmatory test
[a] TA98, TA100 and TA102
[b] Optimal concentration of FDH for detoxification of formaldehyde with TA98
[c] Optimal concentration of FDH for detoxification of formaldehyde with TA100 and TA102
[d] Refer to section 7.2

Results

There was no apparent increase in revertants without S9.
TA98: ≥500 µg/plate poor lawn,
≥2812µg/plate precipitate
TA100:≥ 500 µg/plate poor lawn and at 89µg/plate
TA102:≥158µg/plate

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Formaldehyde clearly showed an increase in revertants both ±S9 as shown in the sponsor's tables. The increase in revertants following exposure to formaldehyde is actually lower in the presence of S9.

Appendix 5 Formaldehyde - Initial Pre-incubation Test in the Absence of S9

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 †	
TA98	Water	0	32	33	19	**28**	8				1.0	
	15.0	0	109	94	96	**100**	8				3.6	+
	20.0	0	101	104	112	**106**	6				3.8	+
	25.0	0	111	118	79	**103**	21				3.7	+
	30.0	0	104	78	96	**93**	13				3.3	+
TA100	Water	0	140	144	124	**136**	11				1.0	
	15.0	0	807	765	746	**773**	31				5.7	+
	20.0	0	1167	1334	1282	**1261**	85				9.3	+
	25.0	0	1142	1126	1158	**1142**	16				8.4	+
	30.0	0	932	796	815	**848**	74				6.2	+
TA102	Water	0	470	469	477	**472**	4				1.0	
	15.0	0	864	827	753	**815**	57				1.7	+
	20.0	0	1023	1097	1091	**1070**	41				2.3	+
	25.0	0	1096	1271	1214	**1194**	89				2.5	+
	30.0	0	1275	1174	1302	**1250**	67				2.6	+

* 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
\+ Substantial dose-related increase in revertant colony counts (fold response ≥2 for TA98 or ≥1.5 for TA100 and TA102)

Appendix 6 Formaldehyde - Initial Pre-incubation Test in the Presence of S9

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 †	
TA98	Water	+	29	30	40	**33**	6				1.0	
	15.0	+	72	76	82	**77**	5				2.3	+
	20.0	+	138	138	135	**137**	2				4.2	+
	25.0	+	104	157	119	**127**	27				3.8	+
	30.0	+	120	106	116	**114**	7				3.5	+
TA100	Water	+	114	114	150	**126**	21				1.0	
	15.0	+	262	311	327	**300**	34				2.4	+
	20.0	+	334	360	281	**325**	40				2.6	+
	25.0	+	217	298	275	**263**	42				2.1	+
	30.0	+	228	181	196	**202**	24				1.6	+
TA102	Water	+	567	582	550	**566**	16				1.0	
	15.0	+	740	610	609	**653**	75				1.2	
	20.0	+	666	593	591	**617**	43	PL	PL	PL	1.1	
	25.0	+	539	533	255	**442**	162	PL	PL	PL	0.8	
	30.0	+	540	533	507	**527**	17	PL	PL	PL	0.9	

* 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
\+ Substantial dose-related increase in revertant colony counts (fold response ≥2 for TA98 or ≥1.5 for TA100)

In the following table of results for clevidipine +S9, the increase in revertants is actually greater than the increase seen for formaldehyde +S9.

Appendix 9 **Clevidipine - Confirmatory Pre-incubation Test in the Presence of S9 (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 †	
TA98	DMSO	+	45	51	35	**44**	8				1.0	
	28.1	+	41	42	42	**42**	1				1.0	
	50	+	45	35	55	**45**	10				1.0	
	89	+	74	68	60	**67**	7				1.5	
	158	+	146	146	167	**153**	12				3.5	+
	281	+	185	190	215	**197**	16				4.5	+
	500	+	166	186	205	**186**	20				4.3	+
	889	+	132	155	156	**148**	14				3.4	+
	1581	+	104	107	115	**109**	6	PL	PL	PL	2.5	+
	2812	+	-	-	-	-	-	ppt IL	ppt IL	ppt IL	-	T
	5000	+	-	-	-	-	-	ppt IL	ppt IL	ppt IL	-	T
TA100	DMSO	+	129	105	113	**116**	12				1.0	
	28.1	+	153	149	157	**153**	4				1.3	
	50	+	178	189	177	**181**	7				1.6	+
	89	+	301	286	286	**291**	9				2.5	+
	158	+	779	727	711	**739**	36				6.4	+
	281	+	1191	1249	1176	**1205**	39				10	+
	500	+	828	766	1009	**868**	126				7.5	+
	889	+	1000	1075	851	**975**	114				8.4	+
	1581	+	478	473	582	**511**	62	PL	PL	PL	4.4	+
	2812	+	-	-	-	-	-	ppt IL	ppt IL	ppt IL	-	T
	5000	+	-	-	-	-	-	ppt IL	ppt IL	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

T 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)

\+ Substantial dose-related increase in revertant colony counts (fold response ≥2 for TA98 or ≥1.5 for TA100)

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Appendix 9 **Clevidipine - Confirmatory Pre-incubation Test in the Presence of S9 (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 †	
TA102	DMSO	+	491	467	447	**468**	22				1.0	
	28.1	+	435	488	435	**453**	31				1.0	
	50	+	468	608	629	**568**	88				1.2	
	89	+	749	753	655	**719**	55				1.5	+
	158	+	1162	1158	1167	**1162**	5				2.5	+
	281	+	2100	2180	2253	**2178**	77				4.6	+
	500	+	2033	2131	2476	**2213**	233				4.7	+
	889	+	2367	2395	2624	**2462**	141				5.3	+
	1581	+	1459	1320	964	**1248**	255				2.7	+
	2812	+	805	958	964	**909**	90	ppt PL	ppt PL	ppt PL	1.9	+
	5000	+	-	-	-	-	-	ppt IL	ppt IL	ppt IL	-	T
WP2 *uvrA*	DMSO	+	37	49	47	**44**	6				1.0	
	28.1	+	47	48	45	**47**	2				1.1	
	50	+	56	64	44	**55**	10				1.2	
	89	+	39	62	69	**57**	16				1.3	
	158	+	63	78	63	**68**	9				1.5	
	281	+	66	71	80	**72**	7				1.6	
	500	+	87	87	98	**91**	6				2.0	+
	889	+	74	78	83	**78**	5				1.8	
	1581	+	73	70	103	**82**	18				1.8	
	2812	+	72	76	87	**78**	8	ppt	ppt	ppt	1.8	
	5000	+	63	65	44	**57**	12	ppt	ppt	ppt	1.3	

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn

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When formaldehyde dehydrogenase was added to the system, there was some decrease in the number of revertants generated, but not total eradication.

Appendix 10 **Clevidipine - Confirmatory Pre-incubation Test in the Presence of S9 and Formaldehyde Dehydrogenase**

Strain	Conc. (µg/plate)	S9 ¶	FDH ‡	Number of revertants x_1	x_2	x_3	mean	SD	Plate observations * x_1	x_2	x_3	Fold response †	
TA98	DMSO[c]	+	+	45	51	35	**44**	8				1.0	
	28.1	+	+	53	44	46	**48**	5				1.1	
	50	+	+	49	45	45	**46**	2				1.1	
	89	+	+	48	64	53	**55**	8				1.3	
	158	+	+	116	111	88	**105**	15				2.4	+
	281	+	+	196	213	158	**189**	28				4.3	+
TA100	DMSO	+	+	158	165	156	**160**	5				1.0	
	28.1	+	+	122	142	164	**143**	21				0.9	
	50	+	+	179	141	196	**172**	28				1.1	
	89	+	+	148	135	161	**148**	13				0.9	
	158	+	+	164	147	121	**144**	22				0.9	
	281	+	+	220	236	216	**224**	11				1.4	
TA102	DMSO	+	+	524	564	498	**529**	33				1.0	
	28.1	+	+	554	526	574	**551**	24				1.0	
	50	+	+	605	517	644	**589**	65				1.1	
	89	+	+	508	529	481	**506**	24				1.0	
	158	+	+	579	558	592	**576**	17				1.1	
	281	+	+	1082	792	907	**927**	146				1.8	+
	500	+	+	1523	1653	1374	**1517**	140				2.9	+
	889	+	+	1274	1233	1221	**1243**	28				2.4	+
	1581	+	+	680	501	747	**643**	127	PL	PL	PL	1.2	

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Addition of FDH to formaldehyde returned the level of revertants essentially to control.

Appendix 13 **Formaldehyde - Confirmatory Pre-incubation Test in the Presence of S9 and Formaldehyde Dehydrogenase**

Strain	Conc. (µg/plate)	S9 ¶	FDH ‡	Number of revertants x_1	x_2	x_3	mean	SD	Plate observations * x_1	x_2	x_3	Fold response †	
TA98	Water	+	+	42	36	54	**44**	9				1.0	
	15.0	+	+	39	30	63	**44**	17				1.0	
	20.0	+	+	70	76	67	**71**	5				1.6	
	25.0	+	+	66	55	73	**65**	9				1.5	
	30.0	+	+	79	71	81	**77**	5				1.8	
TA100	Water	+	+	146	145	136	**142**	6				1.0	
	15.0	+	+	130	154	135	**140**	13				1.0	
	20.0	+	+	131	163	151	**148**	16				1.0	
	25.0	+	+	150	119	126	**132**	16				0.9	
	30.0	+	+	155	170	188	**171**	17				1.2	
TA102	Water	+	+	406	511	453	**457**	53				1.0	
	15.0	+	+	579	530	496	**535**	42				1.2	
	20.0	+	+	560	551	436	**516**	69				1.1	
	25.0	+	+	550	551	564	**555**	8				1.2	
	30.0	+	+	665	737	728	**710**	39				1.6	+

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I agree with the sponsor's evaluation reproduced below:

Initial test:	
	Substantial increases in revertant colony counts were obtained with strains TA98, TA100 and TA102 following exposure to clevidipine in the presence of S9 mix. Substantial increases in revertant colony count were also obtained with the same strains following exposure to the reference article, formaldehyde, except with the strain TA102 in the presence of S9 mix.
Confirmatory test:	
	Substantial increases in revertant colony numbers were obtained with strains TA98, TA100 and TA102 following exposure to clevidipine in the presence of S9 mix. Substantial increases in revertant colony counts were also obtained with the same strains following exposure to the reference, formaldehyde. Visible thinning of the background lawn of non-revertant bacteria and reductions in colony counts were obtained following exposure to clevidipine, indicating that the test article was toxic to the bacteria at the highest levels tested. Precipitation on plates was observed.

While the generation of formaldehyde accounts for some of the genotoxicity, it does not account for all of the effects seen. Does knowing that the drug is metabolized to a genotoxic product make the genotoxicity any less meaningful?

Study title: Mutagenicity evaluation of H324/38 in the L5178Y mouse lymphoma cell thymidine kinase locus mutagenicity test

Key study findings: Substantial increases in mutation frequency were seen at all concentrations in the presence of S9. At the highest concentration the increase exceeded that of the positive control. An increase in revertants was also seen at the two highest concentrations without S9. When the assay was repeated, the mutation frequency with S9 still exceeded that of the positive control. The sponsor repeated the assay again and produced a positive result. The addition of formaldehyde dehydrogenase caused a profound decrease in suspension growth.

Study no.: T2891

Conducting laboratory and location: Astra Safety Assessment, Sweden
Date of study initiation: March 1, 1994
GLP compliance: statement included

QA report: yes (x) no ( )
Drug, lot #, and % purity: batch number 505/93, purity 99.4%

Methods

The assay was performed in 4 parts. The first part was a toxicity study followed by 2 mutagenicity studies ±S9. A third mutagenicity study was performed in which formaldehyde dehydrogenase was added to the cell cultures + test compound +S9.

Growth on the agar was very poor in the first study, necessitating a refinement and repeat.

Clevidipine was degraded in the cell cultures both in the absence and in the presence of the metabolic activation system. The degradation was reported to result in equimolar amounts of H152/81(M1) and formaldehyde. The degradation was reported to be more rapid in the presence of the S9 than without. The addition of formaldehyde dehydrogenase and NAD+ were reported to have no affect on the degradation rate of clevidipine compared to cultures with metabolic activation alone.

Test compound concentrations(mg/l) –S9: 25.4, 50.9,102, 153, 203, 254, 305, 356. At concentrations ≥203 mg/l a precipitate in the culture was reported. Suspension growth as percent of control dropped from 50% at the lowest concentration to 6% at the next concentration.

Test concentrations (mg/ml)+S9: 25.4, 50.9,102, 153, 203, 254, 305, 356. At concentrations ≥203 mg/l a precipitate in the culture was reported. Suspension growth as percent of control was 128% at the lowest concentration, 68% at 102 mg/ml, dropped to 15% at 153 mg/l and 0% at 203 mg/l. .

The sponsor claims that there was no increase in mutation frequency without metabolic activation. However, the appropriate concentration range for evaluation was not studied. The concentrations between 37mg/l and 46.2 mg/l should have been expanded for the correct assessment. This portion of the study is un-interpretable.

Test compound H324/38 Batch 505/93 Positive control 4-nitroquinoline-N-oxide

Concentration of stock solution 0.0405 mol/l Concentration 0.25 mg/l

S9 - 18.5 g/l

Test type		Cell counts x 10^{-4} ml^{-1} Day 1			Day 2			Suspension growth[a]				% of solv control		
		1	2	3	1	2	3	1	2	3	Mean	1	2	3
Solvent control		77.0	77.9	72.9	85.5	88.3	87.9	29.3	30.6	28.5	29.4			
Positive control		43.5	45.2	45.6	55.9	61.5	62.9	10.8	12.4	12.7		37	42	43
Test compound concentration mg/l	mmol/l													
4.62	0.0101	71.2	74.3	70.2	93.4	96.2	87.2	29.6	31.8	27.2		100	108	92
13.9	0.0304	64.3	63.3	75.8	88.3	87.6	80.8	25.2	24.6	27.2		86	84	92
27.7	0.0608	47.6	52.2	43.4	39.5	48.0	54.5	8.4	11.1	10.5		28	38	36
37.0	0.0811	33.0	36.4	38.2	25.6	37.1	43.0	3.8	6.0	7.3		13	20	25
46.2	0.101	30.7	22.6	25.0	22.8	15.8	12.4	3.1	1.6	1.4		11	5	5

The assay with metabolic activation also had a concentration range that should have been expanded for optimum conditions:

139 mg/l: 27-45% of control
162 mg/l: 9-10 % of control
185mg/l: 4-8% of control

However, the mutation frequency increased beyond the level of the positive control.

Substantial increases in the mutation frequency were seen at all tested concentrations.

Study No. 94008 Solvent Dimethyl sulfoxide Start 940322 End 940405

Test compound H324/38 Batch 505/93 Positive control 9,10-dimethyl-1,2-benzanthracene

Concentration of stock solution 0.0405 mol/l Concentration 1.50 mg/l

S9 + 18.5 g/l

Test type		Viable colonies per plate[b]				Plating efficiency[c]			Relative total growth[d] (%)			Mutant colonies per plate[e]			Mutant frequency[f] per 10^6 cells			Mean mutant freq. ± SD
		1	2	3	Mean	1	2	3	1	2	3	1	2	3	1	2	3	
Solvent control		398	415	385	399	108	112	104				149	158	154	139	141	148	142 ± 5
						% of solv control												
Positive control		201	161	159		50	40	40	26	13	13	603	575	555	1110	1321	1792	1241 ± 114
Test compound concentration mg/l	mmol/l																	
92.5	0.203	329	349	326		82	87	82	82	84	66	436	531	593	490	563	673	575 ± 92
116	0.253	182	356	333		46	89	83	32	69	60	462	553	531	939	575	590	701 ± 206
139	0.304	239	249	290		60	62	73	20	17	33	574	517	584	889	768	745	801 ± 77
162	0.355	103	106	122		26	27	31	2.6	2.7	2.8	302	395	328	1085	1379	995	1153 ± 201
185	0.405	1	40	21		0.3	10	5	0.01	0.8	0.3	5	158	114	(1850)*	1443	2009	1726 ± 400

* excluded from the mean

The sponsor then repeated the assay without metabolic activation. A substantial increase in mutation frequency was seen at the highest concentration tested.

Study No. 94008 Solvent Dimethyl sulfoxide Start 940412 End 940425

Test compound H324/38 Batch 505/93 Positive control 4-nitroquinoline-N-oxide

Concentration of stock solution 0.0391 mol/l Concentration 0.25 mg/l

S9 - 17.8 g/l

Test type	Viable colonies per plate[a]				Plating efficiency[c]			Relative total growth[d] (%)			Mutant colonies per plate[e]			Mutant frequency[f] per 10^6 cells			Mean mutant freq. ± SD
	1	2	3	Mean	1	2	3	1	2	3	1	2	3	1	2	3	
Solvent control	335	402	385	374	90.5	109	104				122	128	103	135	118	99.0	117 ± 18
					% of solv control												
Positive control	147	172	175		39	46	47	12	15	15	487	507	471	1226	1091	996	1104 ± 116
Test compound concentration mg/l / mmol/l																	
12.7 / 0.0279	360	379	426		96	101	114	90	105	118	135	108	102	138	105	89.6	111 ± 26
25.5 / 0.0558	382	394	374		102	105	100	80	74	64	127	111	83	123	104	82.1	103 ± 20
35.7 / 0.0781	338	337	335		90	90	90	31	30	23	194	175	130	212	192	144	183 ± 35
45.6 / 0.100	268	241	258		72	64	69	12	10	14	116	132	141	160	203	202	188 ± 24
76.4 / 0.167	143	172	128		38	46	34	3	5	2	104	144	126	269	310	364	314 ± 48

The sponsor then repeated the assay with metabolic activation with a more limited concentration range and again produced a substantial increase in mutation frequency. The MF rate at the highest concentration exceeded that of the positive control.

Test type	Mutant frequency[f] per 10^6 cells			Mean mutant freq. ± SD
	1	2	3	
Solvent control	134	141	124	133 ± 9
Positive control	699	636	634	656 ± 37
Test compound concentration mg/l / mmol/l				
25.5 / 0.0558	137	108	152	132 ± 23
50.9 / 0.112	262	231	245	246 ± 15
76.4 / 0.167	613	573	540	575 ± 37
102 / 0.223	519	427	643	530 ± 109
153 / 0.335	1042	1100	1193	1112 ± 76

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This was repeated again, with a more limited range of concentrations. The results were again unequivocally positive.

Study No. 94008 Solvent Dimethyl sulfoxide Start 940525 End 940606

Test compound H324/38 Batch 505/93 Positive control 9,10-dimethyl-1,2-benzanthracene

Concentration of stock solution 0.0348 mol/l Concentration 1.50 mg/l

S9 + 15.9 g/l

Test type	Viable colonies per plate[b]				Plating efficiency[c]			Relative total growth[d] (%)			Mutant colonies per plate[e]			Mutant frequency[f] per 10^6 cells			Mean mutant freq. ± SD
	1	2	3	Mean	1	2	3	1	2	3	1	2	3	1	2	3	
Solvent control	386	384	407	392	104	104	110				145	129	152	139	124	138	134 ± 8
					% of solv control												
Positive control	192	205	171		49	52	44	28	30	26	561	817	640	1081	1114	1385	1193 ± 167
Test compound concentration mg/l / mmol/l																	
90.7 / 0.199	321	337	326		82	86	83	57	59	57	321	349	302	370	383	343	365 ± 21
102 / 0.224	312	335	288		80	85	73	44	47	40	458	514	476	543	568	612	574 ± 35
125 / 0.273	268	248	302		68	63	77	19	18	22	638	656	604	881	979	740	867 ± 120
136 / 0.298	189	185	194		48	47	49	9	9	9	418	453	491	818	906	936	887 ± 61
147 / 0.323	126	135	129		32	34	33	3	3	3	352	306	379	1034	839	1087	986 ± 131

The sponsor then added formaldehyde dehydrogenase to the incubation mixture. This changed the suspension growth dramatically.

Test compound H324/38 Batch 505/93 Positive control 9,10-dimethyl-1,2-benzanthracene

Concentration of stock solution 0.0348 mol/l Concentration 1.50 mg/l

S9 + 15.9 g/l

Test type	Cell counts x $10^{-4}ml^{-1}$ Day 1			Day 2			Suspension growth[a]				% of solv control		
	1	2	3	1	2	3	1	2	3	Mean	1	2	3
Solvent control	49.7	-	-	79.6	-	-	17.6	-	-	-			
Positive control	35.0	-	-	78.2	-	-	12.2	-	-		69	-	-
Test compound concentration mg/l / mmol/l													
113 / 0.248	47.4	-	-	76.1	-	-	16.0	-	-		91	-	-
125 / 0.273	38.0	-	-	81.5	-	-	13.8	-	-		78	-	-
136 / 0.298	39.2	-	-	84.6	-	-	14.7	-	-		84	-	-
147 / 0.323	38.6	-	-	81.7	-	-	14.0.	-	-		80	-	-
159 / 0.348	35.6	-	-	78.0	-	-	12.3	-	-		70	-	-

While the mutation frequency was profoundly decreased, the comparison is made at a suspension growth that is suboptimal for assessing effects and different from the suspension growth of the cultures without formaldehyde dehydrogenase.

MOUSE LYMPHOMA MUTAGENICITY STUDY 3 - RELATIVE TOTAL GROWTH AND MUTATION FREQUENCY - WITH METABOLIC ACTIVATION AND FORMALDEHYDE DEHYDROGENASE

Study No. 94008 | Solvent Dimethyl sulfoxide | Start 940525 | End 940606

Test compound M324/38 | Batch 505/93 | Positive control | 9,10-dimethyl-1,2-benzanthracene

Concentration of stock solution | 0.0348 mol/l | Concentration | 1.50 mg/l

S9 + | 15.9 g/l

Test type		Viable colonies per plate[b]				Plating efficiency[c]			Relative total growth[d] (%)			Mutant colonies per plate[e]			Mutant frequency[f] per 10^6 cells			Mean mutant freq. ± SD
		1	2	3	Mean	1	2	3	1	2	3	1	2	3	1	2	3	
Solvent control		358	350	348	352	96.8	94.6	94.1				141	135	124	146	143	132	140 ± 7
						% of solv control												
Positive control		222	241	221		63	68	63	43	47	43	563	676	673	938	1038	1127	1034 ± 94
Test compound concentration																		
mg/l	mmol/l																	
113	0.248	373	422	385		106	120	109	96	109	99	137	122	129	136	107	124	122 ± 15
125	0.273	370	382	335		105	109	95	82	85	74	156	152	159	156	147	176	160 ± 15
136	0.298	376	398	334		107	113	95	90	95	80	166	140	147	163	130	163	152 ± 19
147	0.323	348	365	395		99	104	112	79	83	90	174	144	134	185	146	126	152 ± 30
159	0.348	422	380	375		120	108	106	84	76	74	114	116	121	100	113	119	111 ± 10

The sponsor concluded that there was a concentration-related increase in mutation frequency at concentrations of≥167mmol/l. The sponsor further concluded that the addition of formaldehyde dehydrogenase and nicotinamide adenine dinucleotide (NAD+) abolished the mutagenic activity seen under metabolic activation conditions in the same concentration range.

Study title: Mutagenicity evaluation of formaldehyde in the L5178Y mouse lymphoma cell thymidine kinase locus mutagenicity test

Key study findings: This study provides historical data to support the mutagenic effects of formaldehyde in the test system.

Study no.: t2886

Conducting laboratory and location: Astra Safety Assessment, Sweden
Date of study initiation: January 9, 1985
GLP compliance: no statement
QA report: yes ( ) no (x)

Drug, lot #, and % purity: formaldehyde, batches 32761399 and 446K4964703, purity 37%

Methods This study was apparently conducted 10 years prior to the mouse lymphoma study for clevidipine.

Study No. 53 Solvent Fischer's medium (F_{GD}) Start 850129 End 850211

Test compound Formaldehyde Batch 327261399 Positive control 2-nitrofluorene

Concentration of stock solution 0.0290 mol/l Concentration 50 mg/l

S9 - 0.671 g/l

Test type	Cell counts x $10^{-4}ml^{-1}$ day 1	day 2	Susp. growth[a]	Viable colonies per plate	Mean[f]	Plating effic.[b]	Total growth[c] %	Mutant colonies per plate	Mean[e]	Mutant frequency[d] per 10^6 cells
Solvent control	66.7	60.2	18.1	313,326,304	314	92.5	-	60, 63, 46	56.3	60.9
Solvent control	65.5	55.1	16.5	305,326,307	313	92.0	-	74, 70, 86	76.7	83.4
			% of solv control			% of solv control				
Positive control	38.9	57.8	56	218,206,220	215	68	38.4	223,225,213	220	349
Test compound concentration mg/l / mmol/l										
4.20 / 0.140	23.1	65.6	38	140,137,150	142	45	17	378,383,334	365	874
5.10 / 0.170	23.1	64.4	37	129,125,128	127	41	15	290,297,293	293	785
6.01 / 0.200	22.2	35.6	21	79, 94, 72	81.7	26	5	182,192,179	184	767
6.91 / 0.230	19.1	25.3	13	84, 87, 85	85.3	27	4	231,211,194	212	845
7.80 / 0.260	21.3	17.1	10	44, 43, 48	45.0	14	1	145,161,151	152	1150

a=(day 1/day 0) x (day 2/day 1 diluted) c=(relative suspension growth X relative plating efficiency) / 100

b=$\frac{\text{mean viable colonies}}{\text{number of cells seeded}}$ x 100 d=$\frac{e}{f}$ x dilution (3.4×10^{-4}) e = factor

This study provides historical data to support the mutagenic properties of formaldehyde in this test system. Is it reassuring to know that clevidipine is metabolized to a known mutagen?

Study title: Lymphocyte transformation test (LTT) with clevidipine (H324/38) in human lymphocytes in vitro.

Key study findings: The sponsor concluded that the study supported the hypothesis that increases in mitotic indices observed in a chromosome aberration assay were due to mitotic arrest and not to any stimulatory properties of clevidipine on human lymphocytes.

Study no.: t3363

Conducting laboratory and location: Not sure. Astra Safety Assessment, Sweden
Date of study initiation: July 1, 1996
GLP compliance: no statement found
QA report: yes ( ) no ( )
Drug, lot #, and % purity: clevidpine batch 300/94, 99.6% purity

Methods

The sponsor states the purpose for this study as follows:

> Clevidipine (H324/38), a short-acting Ca^{2+} channel blocking agent, was tested for its ability to induce DNA-synthesis in human lymphocytes. This study was initiated since H324/38 was shown to increase the mitotic index in a chromosome aberration test with human lymphocytes.

The drug did more than just increase the mitotic index. In report t3376/study 95126,"Analysis of structural chromosme aberrations in human lymphocytes treated with clevidipine in vitro", the sponsor concluded that :

> In cultures without metabolic activation a small but statistically significant increase in the frequency of aberrated cells was found at the highest concentration. However, in cultures with metabolic activation, a substantial increase was seen at both the intermediate and at the high concentration. The increase was dose related.

DNA synthesis was measured using a lymphocyte transformation test (LTT). Lymphocytes were incubated with test compound and ^{3}H-thymidine. At the end of the incubation the cells were harvested on glass fiber filters and ^{3}H-thymidine not bound to DNA was removed by washing. The radioactivity on the filters gives a measure that is directly proportional to the DNA synthesis activity. Prior to addition of the test compound, the cell cultures were incubated with phytohemagglutinin to stimulate the cells to replicate. The test articles were added after 46 hours of incubation and remained present until addition of ^{3}H-thymidine followed by harvest. The cells were sampled after 72 hours for the first harvest and after 96 hours for the second harvest. In addition, cultures of human lymphocytes were treated with H324/38, without prior incubation with PHA, to reveal any stimulation by the drug itself.

The test compound was tested at the same concentration as used in the chromosome aberration assay without metabolic activation: 0.08, 0.16 and 0.32 mmol/l. Six replicate cultures were used for each test concentration and control. Radioactivity was expressed as counts per minute (cpm).

The results were presented essentially as the raw data of CPM followed by various comparisons which are not entirely clear to me.

Appendix 2: Statistical analysis

Culture	Equal to
A (n=6)	Control with culture medium only
C (n=6)	Control with PHA and solvent
D (n=6)	0.32 mM H324/38 and PHA
E (n=6)	0.16 mM H324/38 and PHA
F (n=6)	0.08 mM H324/38 and PHA
G (n=6)	0.16 mM H324/38
H (n=6)	0.08 mM H324/38

Differences observed between the cultures expressed as logcpm

Contrast	Subject No. 1				Subject No. 2				Subject No. 3				Subject No. 4			
	Harvest I	p-value	Harvest II	p-value	Harvest I	p-value	Harvest II	p-value	Harvest I	p-value	Harvest II	p-value	Harvest I	p-value	Harvest II	p-value
C minus D	4,3	0,0001	5,09	0,0001	4,36	1E-04	4,87	0,0001	4,12	1E-04	4,41	0,0001	5,94	0,0001	5,2	0,0001
C minus E	0,66	0,0015	1,44	0,0001	1,08	1E-04	1,26	0,0001	0,47	0,02	0,98	0,0001	2,03	0,0001	2,05	0,0001
C minus F	0,18	0,36	0,76	0,008	0,13	0,61	0,55	0,0019	0,04	0,85	0,3	0,18	0,23	0,38	0,32	0,25
A minus G	0,52	0,01	1	0,0007	0,23	0,34	1,32	0,0001	-4,4	1E-04	0,55	0,018	0,21	0,42	0,09	0,73
A minus H	0,51	0,007	0,51	0,068	0,7	0,006	0,9	0,0001	1,45	0,16	0,9	0,0002	0,61	0,02	0,55	0,05

The sponsor came to the following conclusion:

> It is concluded that H324/38 does not induce increased DNA synthesis in human lymphocytes, either following PHA stimualtion or by itself, under the current in vitro conditions.
>
> Thus, the results from this study supports the suggestion that the increases in mitotic indeces observed in the chromosome aberration assay was most likely due to a mitotic arrest and not to any stimulatory properties of H324/38 on human lymphocytes.

2.6.6.5 Carcinogenicity

Carcinogenicity studies not required due to the short term use of the drug.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: ***H324/38: Continuous intravenous infusion fertility and early embryonic development study in the female rat***

Key study findings: The primary findings were the number of clevidipine-treated females who were pseudopregnant and/or had estrus cycles of irregular duration.

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

Study no./report no.: 97049
Experimental start date: sometime in 1997
Study location: Astra, Charnwood, UK
Test article: 200/96, purity 99.7%
GLP: statement included
QA: yes

Groups of 19 female rats were dosed by continuous IV infusion with 29, 77 and 121 μmol/kg/day (13, 35 and 55 mg/kg/day) clevidipine. An additional group of 18 female rats was dosed with vehicle, Intralipid 20%. All doses were given at the same infusion rate for at least 14 days prior to mating, throughout mating and up to Day 7 post-coitum. Females were mated with untreated males.

Animals were cannulated prior to start of the study. During the study, animals were checked daily for signs.
Bodyweights were recorded day of surgery and once weekly until mated then on days 0,3,6,9, 12 and 14 post-coitum. Food consumption was monitored continuously between days 1-8d and days 8 -15d then after mating between days 0-14 post-coitum.
Estrus cycle monitoring was performed. Very little detail was provided in the text.

Animals euthanized or dying ahead of schedule were necropsied and macroscopic observations made. Scheduled euthanasia was day 14 post-coitum.

Results

Body Weight

There was no consistent effect in the data presented.

Table 4: Group Mean Pregnant Female Bodyweights (g)

Dose Group	Group Mean Bodyweight (g) ± Standard Deviation									
	Day 1d	Day 8d	Day 15d	Day 22d	Day 0 *pc*	Day 3 *pc*	Day 6 *pc*	Day 9 *pc*	Day 12 *pc*	Day 14 *pc*
1	239.1 ± 24.2	247.6 ± 25.3	253.7 ± 30.2	254.5 ± 18.9	256.3 ± 30.6	274.8 ± 32.8	287.1 ± 32.9	291.6 ± 31.6	311.2 ± 33.7	317.6 ± 36.8
2	236.7 ± 20.4	250.2 ± 23.0	253.4 ± 21.4	262.3 ± 33.7	255.7 ± 22.2	276.9 ± 24.5	285.0 ± 25.3	292.7 ± 27.5	304.5 ± 26.5	314.8 ± 28.4
3	237.6 ± 21.3	247.6 ± 21.4	252.5 ± 21.2	255.0 ± 33.3	253.4 ± 21.5	272.6 ± 22.6	284.0 ± 24.6	286.6 ± 21.2	301.0 ± 22.8	305.1 ± 26.3
4	243.0 ± 19.2	253.6 ± 15.1	261.3 ± 17.1	277.0 ± 9.3	263.4 ± 18.1	281.1 ± 16.8	292.3 ± 19.1	299.5 ± 19.7	316.6 ± 20.1	324.4 ± 21.9

d : Days of dosing prior to mating
pc : Days of dosing *post-coitum*

group	Change Day 1-22	Change Day 1-14
1	15.4	61.3
2	25.6	59.1
3	17.4	51.7
4	34	61

There were no obvious changes in food consumption.

A few animals from all the clevidipine groups were or became pseudopregnant at the start of dosing. All of these animals were reported to become cyclic and mate within 4 pairings. There were also a number of clevidipine-treated animals who had cycles of unusual duration(<4 days or >5days) These animals were also reported to mate within 4 pairings. None of the control animals were pseudopregnant or had unusual cycle duration. The sponsor's summary of these findings is shown below.

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

The sponsor's tabular summary is shown below.

Study Title: Clevidipine: Continuous Intravenous Infusion Fertility and Early Embryo-Foetal Development Study In The Female Rat				Study Number 97049
Location: Astra Safety Assessment				Date: 1997
Compound: Clevidipine	Administration Route: Intravenous Infusion		Frequency: Continuous	
Test Formulation: Clevidipine in 20% lipid emulsion			Batch Number: HL-012 (Dose Group 2) HL-013 (Dose Group 3) HL-014 (Dose Group 4)	
Control Formulation: 20% Intralipid®			Batch Number: 86426-51 86345-51 87060-51	
Species: Rat Strain: Sprague Dawley	Age at Start of Dosing: 10 to 18 Weeks		Bodyweight at Start of Dosing: 201 to 292 g	
Day of Mating designated Day 0 *post-coitum*	Treatment of Females for min of 2 weeks pre-mating until Day 7 *post-coitum*		Caesarian Section on Day 14 *post-coitum*	

Dose Group	1	2	3	4
Dose of $\mu mol \cdot kg^{-1} \cdot day^{-1}$	Vehicle Control	29	77	121
$mg \cdot kg^{-1} \cdot day^{-1}$		13	35	55
Number of Females Mated	18	20	20	19
Number Pregnant	18	20	20	17[a]
Mortalities	0	0	0	1
This study was carried out in compliance with GLP				
Findings (Implantation Data)				
Number of Total Intrauterine Loss	1	0	1	0
Number of Viable Litters	17	20	19	17
Mean Number of Corpora Lutea	14.4	13.7	13.2	14.4
Mean Number of Implantations	13.1	12.4	10.9	12.5
Mean Number of Foetuses	11.3	11.8	9.4	11.4
Mean % per Litter Pre-Implantation Loss	10.6	11.2	18.4	12.8
Mean % per Litter Post-Implantation Loss	10.6	4.9	16.6	8.6

a: Pregnancy status of animal which died pre-term was not discernible

Study title: ***H324/38: Continuous intravenous infusion dose finding embryo-fetal toxicity study in the rat***

Key study findings: I agree with the sponsor's conclusion that the MTD is somewhere between 85 and 145µmol/kg/day.

Study no./report no.: 96126-1
Experimental start date: August 15, 1996
Study location: Astra, Charnwood, UK
Test article: BatchesAH-006, HL-004, AS-008
GLP:no

Groups of 5 mated female rats were dosed by continuous infusion with either 50, 85 or 145 µmol/kg/day (23,39 or 66 mg/kg/day) from day 6 to day 16 post-mating. An additional group of 4 mated rats was dosed with the control substance, saline and 5 mated animals were dosed with the vehicle, 20% Intralipid. All doses were given at a rate of 0.92 ml/kg/hr.

One MD was replaced day 8 due to loss of cannula patency
One HD was replaced day 8 due to loss of cannula patency
Two LD animals showed ill health including periarticular swelling and lameness. At necropsy, both showed evidence of infection. One was not pregnant and the other had a high proportion of intrauterine deaths.

Signs of decreased-absent feces, piloerection, staining of the head/nostrils, stained anogenital region, and mucoid vaginal discharge were restricted to the HD group.

The Intralipid control group, the LD and MD drug-treated groups gained on average more weight than the saline control group. The HD group lost weight. The sponsor's summary is shown below.

Table 3: Mean Pregnant Bodyweights

Dose Group	Bodyweight (g) on Day Number *post-coitum*							Adjusted Bodyweight	Bodyweight Gain
	0	6	9	12	15	18	21	Day 21[a]	Day 6 to 21[a]
1	278.8	306.8	321.5	336.0	350.0	378.5	418.0	319.5	12.8
	11.1	11.7	16.4	21.2	24.8	19.8	24.3	22.8	15.2
2	278.6	303.4	309.6	326.2	339.6	370.6	422.6	326.6	23.2
	18.0	15.3	13.7	14.0	14.1	18.5	17.4	10.4	7.1
3	283.0	296.7	307.3	323.3	340.7	376.3	433.3	318.8	22.2
	12.8	18.9	24.7	12.5	22.7	28.7	36.5	19.8	9.1
4	274.0	310.8	320.0	327.8	347.3	374.3	423.3	331.8	21.1
	10.6	5.6	12.2	10.4	10.9	12.6	18.8	9.2	9.8
5	271.3	293.7	304.0	295.0**	284.3***	270.0***	324.0***	256.5***	-37.0***
	8.0	4.0	7.0	18.7	5.0	19.7	7.2	7.8	5.6

n: Mean
n: Standard Deviation
a: Bodyweight adjusted for gravid uterus weight
**: $p < 0.01$
***: $p < 0.001$

Food consumption decreased significantly for the Intralipid group and the drug-treated groups. The HD group decreased food consumption by 56% compared to the saline control group.

Macroscopic necropsy findings included the evidence of infections, enlarged lymph nodes, adrenals, spleen and pale kidneys.

Table 5: Summary of Maternal Macroscopic Necropsy Findings (Number of Animals Affected)

Observation		Dose Group				
		1	2	3	4	5
Number of Animals		4	5	3	5	5
Cannula Tip	Small amount of pus	-	1	-	-	2
Lymph Nodes	Enlarged	-	3	3	1	1
Kidney	Increased Pelvic Cavitation	-	-	1	1	-
Kidney(s)	Pale Areas	-	-	-	1	1
Kidney	Enlarged	-	-	-	-	1

Day 6 *post-coitum*		Days 6 to 16 *post-coitum*		Day 21 *post-coitum*		
Dose Group		1	2	3	4	5
Dose of H 324/38	$\mu mol \cdot kg^{-1} \cdot day^{-1}$	Control Saline	Vehicle 20% Intralipid	50	85	145
	$mg \cdot kg^{-1} \cdot day^{-1}$			23	39	66
Number of Females Mated		4	6[a]	6[a]	5	5
Number Pregnant at Day 21 *post-coitum*		4	5	4[b]	4	3
Mortalities		0	0	0	0	0
This study was carried out in compliance with GLP						
FINDINGS (General)						
Dose Group		1	2	3	4	5
Bodyweight Gain Adjusted For Gravid Uterus Weight (g) Day 6 to 21 *post-coitum*		12.8	23.2	22.2	21.1	-37.1
Number of Pre-Term Terminations while Pregnant		0	1	1	0	0
Number of Total Intrauterine Deaths		0	0	0	0	0
Number of Viable Litters		4	5	4[b]	4	3
Mean Number of Corpora Lutea		14.75	14.80	17.00	15.50	14.67
Mean Number of Implantations		14.00	14.40	16.33	12.50	13.00
Mean Number of Live Foetuses		13.3	13.6	15.7	12.3	11.0
Mean % per Litter of Early Intrauterine Deaths		4.73	5.18	4.17	3.13	12.90
Mean % per Litter of Late Intrauterine Deaths		0	0	0	0	2.37
Mean % per Litter of Dead Foetuses		0	0	0	0	0
Mean % per Litter Pre-Implantation Loss		5.05	2.72	3.93	18.98	10.57
Mean % per Litter Post-implantation Loss		4.73	5.18	4.17	3.13	15.27
Mean Weight of Male Foetuses (g)		5.77	5.30*	5.50	5.73	4.58***
Mean Weight of Female Foetuses (g)		5.47	5.10	5.22	5.44	4.24**
Sex Ratio (%) Males: Females		56.6:43.6	48.5:51.5	53.2:46.8	59.2:40.8	48.5:51.5

a: One animal terminated prematurely due to loss of cannula patency and replaced (continued)
b: One Dose Group 3 animal excluded from data analysis due to infection
*: $p < 0.05$
**: $p < 0.01$
***: $p < 0.001$

I agree with the sponsor's conclusion that the MTD is somewhere between 85 and 145µmol/kg/day.

Study title: H324/38: ***Continuous intravenous infusion dose finding embryo-fetal toxicity study in the Dutch rabbit***

Key study findings: Given the limited number of animals and the lack of unequivocal findings, I reservedly agree with the sponsor's conclusion that the maximum dose to be used for the definitive embryo-fetal toxicity study should be between 87 and 145 μmol/kg/day.

Study no./report no.: 96160-1
Experimental start date: November 4, 1996
Study location: Astra, Charnwood, UK
Test article: Batches AS-010, HL-004, HL-005, AS-008
GLP: statement included
QA: yes

Groups of 5,5 and 4 mated female Dutch rabbits were dosed with clevidipine by continuous intravenous infusion from day 6 to 19 post-mating. Doses used were 73, 87 and 145 μmol/kg/day (33, 40 and 66 mg/kg/day). An additional group of 5 mated rabbits were given the 20% Intralipid vehicle.

Bodyweights and food consumption were recorded regularly. Animals were also monitored for clinical chemistry parameters.

Results

Dose-related signs of maternal toxicity included decreased food consumption and feces production.

Observation	Dose Group 1	Dose Group 2	Dose Group 3	Dose Group 4
Reduced Food Consumption (> 3 days)	0/5	2/5	3/5	3/5
Reduced Faecal Production (> 3 days)	3/5	2/5	3/5	4/4

Two - 4 animals in each group did not produce a viable litter at necropsy either due to the cannula, abortion, total litter loss or not being pregnant.

There were no consistent effects on body weight.

Table 4: Pregnant Female Bodyweight

Dose Group	Bodyweight (kg) on Day Number *post-coitum*								Adjusted [a] Bodyweight (kg)	Bodyweight Change Day 6 to 18 *post-coitum* (kg)
	0	6	8	10	14	18	22	29		
1	2.28	2.23	2.20	2.27	2.23	2.10	2.20	2.45	2.17	-0.17
	± 0.30	± 0.28	± 0.24	± 0.32	± 0.38	± 0.35	± 0.28	± 0.35		± 0.06
2	2.08	1.98	1.95	1.90	1.87	1.83	2.15	2.30	2.26	-0.27
	± 0.22	± 0.26	± 0.24	± 0.22	± 0.31	± 0.32	± 0.35	± 0.28		± 0.23
3	2.14	2.10	2.12	2.15	2.10	2.03	2.13	2.33	1.97	-0.13
	± 0.19	± 0.16	± 0.16	± 0.10	± 0.00	± 0.05	± 0.13	± 0.10	± 0.12	± 0.17
4	2.25	2.10	2.10	2.15	2.10	2.00	2.00	2.40	2.19	-0.10

a: Bodyweight at necropsy adjusted for gravid uterus weight

The clinical chemistry data was presented as single animal values. Given the limited number of animals surviving for sampling, it is very difficult to discern effects from natural variability in the data. Increased plasma triglycerides, cholesterol, total bilirubin, AST and ALT were attributed to the vehicle.

Dose Group		1	2	3	4
Dose of	$\mu mol \cdot kg^{-1} \cdot day^{-1}$	Vehicle	73	87	145
Clevidipine	$mg \cdot kg^{-1} \cdot day^{-1}$	Vehicle	33	40	66
Infusion Rate ($ml \cdot kg^{-1} \cdot h^{-1}$)		0.92	0.46	0.92	0.92
Number of Females Mated		5	5	5	4
Number of Females Pregnant		4	4	5	2[a]
Number Killed Prematurely: Pregnant		1[b]	1[c]	1[b]	0
Number Killed Prematurely: Not pregnant		1[b]	0	0	0
This study was carried out in compliance with GLP					
FINDINGS					
Dose Group		1	2	3	4
Number of Abortions		1	1	0	1
Number with Total Litter Loss		0	1	1	0[a]
Number of Viable Litters		2	1	3	1
Mean Number of Corpora Lutea per Litter		7.0	8.0	9.8	9.0
Mean Number of Implants per Litter		6.5	5.5	9.8	8.0
Mean Pre-Implantation Loss (% per litter)		8.4	27.8	0.0	11.1
Mean Number of Live Foetuses per Litter		6	4	9	7
Mean Placental Weight (g)		4.76	5.28	3.57	4.30
Mean Number of Early Intrauterine Deaths (% per litter)		6.3	50.0	27.3	12.5
Mean Number of Late Intrauterine Deaths (% per litter)		0.0	0.0	2.3	0.0
Mean Number of Dead Foetuses (% per litter)		0.0	0.0	2.5	0.0
Mean Weight of Male Foetuses (g)		33.8	41.6	27.2	32.4
Mean Weight of Female Foetuses (g)		32.6	45.2	26.0	32.1
Sex Ratio (%) Males : Females		50.0 : 50.0	50.0 : 50.0	59.3 : 40.7	42.9 : 57.1
Maternal Findings (Number of animals affected)					
Reduced Faecal Production (> 3 days)		3/5	2/5	3/5	4/4
Reduced Food Consumption (> 3 days)		0/5	2/5	3/5	3/4
Excessive Mammary Tissue at Necropsy		0/5	0/5	0/5	1/4

a: In addition, 2 had uncertain pregnancy status (may have been very early litter loss)
b: Animal killed due to swelling under chin; cannula dislodged from vessel
c: Animal killed due to severe skin inflammation dorsal surface and alopecia

Study title: H324/38: ***Continuous intravenous infusion embryo-fetal toxicity study in the rat***

Key study findings: Animals were dosed to the point of toxicity with the HD group gaining 30% less than the control group. Soft tissues findings in pups: dose-related increase in litters affected with renal pelvic cavitation. The findings were statistically significant at the MD and HD groups. I didn't see a strong signal for skeletal findings but the sponsor took a very conservative interpretation of a MD litter with tail and limb anomalies and other litters with malrotations of a hindlimb.

Study no./report no.: 97003
Experimental start date: November 4, 1996
Study location: Astra, Charnwood, UK
Test article: Batch200/96, purity 99.7% however the formulations listed 3 other batches: HL006, HL007, HL008
GLP: statement included
QA: yes

Methods

Groups of 23 mated female rats were dosed by continuous IV infusion with either 29, 77 or 121 μmol/kg/day (13, 35 or 55 mg/kg/day) of clevidipine from GD6to GD17. Additional groups of 24 mated rats were dosed with the vehicle of 20% Intralipid. Animals receiving the highest dose had the cannulas flushed each day to reduce precipitation of the compound in the cannula.

Signs: daily
Bodyweights: once weekly until mated, GD0, 3,6,9,12,15,18 and 21
Food consumption: continuous from GD6-GD21.
Animals were euthanized day 21 and observations made for intrauterine deaths, empty implantation sites, fetal viability. Corpora lutea were counted.

Fetuses were euthanized and examined for skeletal anomalies or soft tissue findings (about half of each litter). The sponsor described the scale used to assess the ossification of bones in the paws.

Additional animals were used for TK sampling: 2 treated with vehicle, 2 treated with 77μmol/kg/day and 4 each treated with 29 and 121 μmol/kg/day. Dosing was similar to that used for the main study. Plasma values were used to confirm exposure to drug.

Results

Unscheduled mortality was seen.
1 control: found dead GD20 due to infection
2 HD: died, euthanized prematurely; impacted intestines

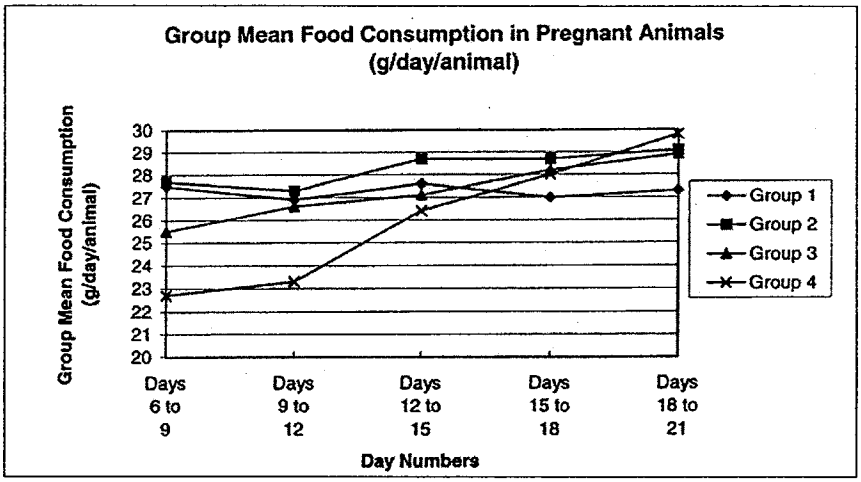
Plasma analysis confirmed increasing levels of the metabolite (H152/81) with increasing doses of drug (see table below).

The sponsor's summary of findings shown below indicates that the rats were dosed to the point of toxicity with regard to clinical observations.

FINDINGS (Maternal Observations Worthy of Note)

Dose Group	1	2	3	4
Toxicokinetics (Concentration of H152/81 in Blood Plasma)				
Median Value ($\mu mol \cdot l^{-1}$)	<0.1	83.0	130	178
Range of Values ($\mu mol \cdot l^{-1}$)	-	45.4-120	99.2-164	153-263
Clinical Signs (Number of Animals Affected)				
Hunched Posture	-	-	-	2
Firm Abdomen	-	-	-	2
Cold/Pale Extremities	-	-	-	2
Depressed Activity/Disinclined to Move/Subdued	-	-	-	3
Piloerection	1	-	2	7
Eye(s) Half Shut	-	3	2	1
Blood in Cage/ Brown Discharge from Vagina	1	1	2	3
No and/or Reduced Faecal Production/Dark Faeces	-	-	1	6
Greasy Fur	-	-	1	4
Group Mean Maternal Food Consumption ($g \cdot day^{-1}$ per animal)				
Days 6 to 9 *post-coitum*	27.5	27.7	25.5*	22.7***
Days 9 to 12 *post-coitum*	26.9	27.3	26.6	23.3***

The food consumption data would be more meaningful with the inclusion of food consumption prior to commencement of dosing. It appears that the HD showed a marked decrease in food consumption in the first 3 days of dosing and then returned to levels of consumption similar to the other groups.



The HD group also gained less body weight than did the other groups (~30% less gain than the control group), another indication of dosing to the point of toxicity.

Table 5: Group Mean Pregnant Female Bodyweights (Excluding Satellites)

Dose Group	Group Mean Bodyweight (g) ± Standard Deviation								Adjusted Bodyweight[a]	Bodyweight Gain[b]
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21		
1	241.8	258.2	270.2	281.1	296.3	312.0	340.6	385.8	294.4	23.6
	± 21.4	± 19.7	± 22.3	± 23.1	± 25.2	± 30.3	± 35.9	± 41.5	± 32.1	± 13.5
2	246.3	265.0	275.3	288.5	305.9	320.9	348.3	395.2	300.5	25.2
	± 16.7	± 15.8	± 15.0	± 16.7	± 17.3	± 21.5	± 23.4	± 25.1	± 16.8	± 8.6
3	239.9	258.9	270.0	280.9	296.6	311.1	340.1	376.4	296.6	26.7
	± 16.4	± 17.4	± 19.0	± 20.5	± 23.4	± 27.3	± 35.4	± 42.8	± 28.1	± 17.0
4	239.6	257.2	267.7	274.6	287.0	302.3	328.7	372.3	283.2	16.5
	± 15.2	± 15.2	± 17.3	± 16.1	± 15.3	± 19.2	± 21.8	± 27.7	± 18.6	± 18.8

a: Day 21 *post-coitum* bodyweight adjusted for gravid uterus weight
b: Bodyweight gain between Day 6 *post-coitum* and Day 21 *post-coitum* using adjusted bodyweight

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ON ORIGINAL

Soft tissue findings included renal pelvic cavitation and absence of renal papilla.

Observation	Total Number of Foetuses Affected (Number of Litters Affected) % of Foetuses Affected			
	Dose Group 1	Dose Group 2	Dose Group 3	Dose Group 4
Total Number of Foetuses Examined	147	151	110	133
Ureter(s): Slight increased dilatation	6 (6) 4%	7 (6) 5%	9 (6) 8%	6 (5) 5%
Ureter(s): Moderate increased dilatation	4 (3) 3%	5 (4) 3%	6 (4) 5%	2 (2) 2%
Ureter(s): Extreme increased dilatation	1 (1) 1%	1 (1) 1%	0 (0) 0%	1 (1) 1%
Kidney(s): Slight increased renal pelvic cavitation	2 (1) 1%	6 (4) 4%	9 (5) 8%*	12 (8) 9%**
Kidney(s): Moderate increased renal pelvic cavitation	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 1%
Kidney: Absence of renal papilla	1 (1) 1%	1 (1) 1%	1 (1) 1%	5 (3) 4%

*: $p<0.05$
**: $p<0.01$

Using the litter incidence of various skeletal findings, there was no particular signal for a drug effect. However, the sponsor took a very conservative interpretation of the data and cited a MD litter which had fetuses with tail and limb anomalies. They also cite other litters with malrotations of a hindlimb and some positional changes. Decreased uterine blood flow can affect digits and tails in rat fetuses and such anomalies have been reported with other calcium channel blockers. Part of the sponsor's summary regarding these findings is shown below.

> The Dose Group 3 litter which had foetuses with tail and limb anomalies, also had a high post-implantation loss which suggests that these effects were possibly related to maternal toxicity. However, calcium antagonists can also reduce uteroplacental blood flow[2]. Mechanical vascular clamping of the uterine blood flow can result in embryonic loss, digit anomalies and shortened tails in rat foetuses[2]. After maternal treatment with calcium antagonists, kinked tails were seen with Clentiazem[3] and shortened tails and digit anomalies with Diproteverine[4] in rat foetuses. So, it cannot be ruled out that these effects were related to clevidipine treatment.
>
> The slight malrotations of a hindlimb seen in three Dose Group 2 foetuses and two Dose Group 3 foetuses were minor positional changes. In the proportion of the foetuses from each dose group which were examined skeletally, these changes were not due to skeletal alterations and was also seen in one control foetus. One of the affected foetuses, from the Dose Group 3 litter with tail anomalies, also had bilateral forelimb flexure. This finding is which is also a positional change and was not due to any skeletal alteration. The significance of these findings is uncertain.

Dose Group		1	2	3	4
Dose of	$\mu mol \cdot kg^{-1} \cdot day^{-1}$	Vehicle Control	29	77	121
	$mg \cdot kg^{-1} \cdot day^{-1}$		13	35	55
Number of Females Mated[a]		24	23	23	23
Number Pregnant[a]		24	23	20	23
Mortalities[a]		1	0	0	2
This study was carried out in compliance with GLP					
Findings (Implantation Data)[b]					
Number of Total Intrauterine Deaths		1	0	0	0
Number of Viable Litters		22	23	20	21
Mean Number of Corpora Lutea		14.48	14.91	13.95	14.33
Mean Number of Implantations		13.37	13.78	12.65	13.00
Mean Number of Live Foetuses		13.3	13.2	10.8**	12.7
Mean % per Litter of Early Intrauterine Deaths		3.19	3.95	14.53	2.45
Mean % per Litter of Late Intrauterine Deaths		0	0	0.05	0
Mean % per Litter of Dead Foetuses		0	0	0	0
Mean % per Litter Pre-Implantation Loss		3.98	6.67	7.50	9.47
Mean % per Litter Post-Implantation Loss		3.19	3.95	14.91	2.45
Mean Weight of Male Foetuses (g)		5.52	5.51	5.45	5.29
Mean Weight of Female Foetuses (g)		5.24	5.17	5.08	5.02
Sex Ratio (%) Males: Females		49:51	49:51	47:53	45:55

a: Excluding satellite animals
b: Excluding total intrauterine loss
**: P<0.01

Prenatal and postnatal development

Study title: ***A continuous intravenous infusion male fertility study of clevidipine in the rat.***

Key study findings: Under the conditions of the study, at doses up to 55 mg/kg/day there were no findings of adverse drug effects upon the measured parameters of fertility and early embryonic development. Male parameters assessed included sperm motility, morphology and count. A search of the appendices revealed that sperm morphology had been evaluated but was not summarized in the body of the report. There were no apparent effects on sperm morphology. There is nothing in the report to suggest any special consideration given to the histological evaluation.

Study no.: 900594
Conducting laboratory and location:

Date of study initiation: dosing started January 17, 2005
GLP compliance: statement included
QA reports: yes (x) no ( )
Drug, lot #, and % purity: KV1348 in a 20% lipid emulsion

Methods
Males were dosed for 28 days prior to placement for mating, during mating and until study day 39 for males that had successfully mated or study day 44 for males not having mated. Total length of treatment was therefore 39 to 44 days.
Animals were observed daily for signs. Body weight and food consumption were measured twice weekly.

Mated females were weighed on days 0, 3, 7, 10 and 13 of gestation. The estrous cycle of all females were determined for at least 10 days before placement for mating and during mating until positive identification of mating.

Each treated male was placed for mating with an untreated female for a maximum of 14 days. Gestation day 13, females were euthanized and gross pathological examination of the reproductive tract was performed. Ammonium sulfide was used for determination of implantation sites. The corpora lutea were counted, the uterine contents examined, numbers of live and dead embryos and resorptions recorded. Terminal evaluations for males included gross pathology, organ weights (epididymides, prostate, seminal vesicles, and testes), testicular histopathology, sperm motility, sperm morphology and spermatozoa count.

Cannulas were surgically implanted prior to the start of the study. A jacketed tether system was used. The infusion rate was 0.92 ml/kg/hour. The first day of dosing was designated as Day 1.

Results

Unscheduled mortality was seen. The following males were either euthanized or died during the study.

Vehicle control: 2 males
13 mg/kg/day: 7 males
35 mg/kg/day: 9 males
55 mg/kg/day: 2 males

The males listed above showed decreased activity, abnormal respiration(undefined), coldness, decreased muscle tone, dehydration. Males with abdominal distension also had enlarged testes. These males also showed gross changes at the infusion site of swelling, thickening and/or masses. Findings in the lungs were mottled, pale or dark foci, dark areas, discoloration and raised or depressed areas. The sponsor reported these as indicative of both vehicle and dose-dependent clevidipine effects.
The sponsor's study design summary is shown below.

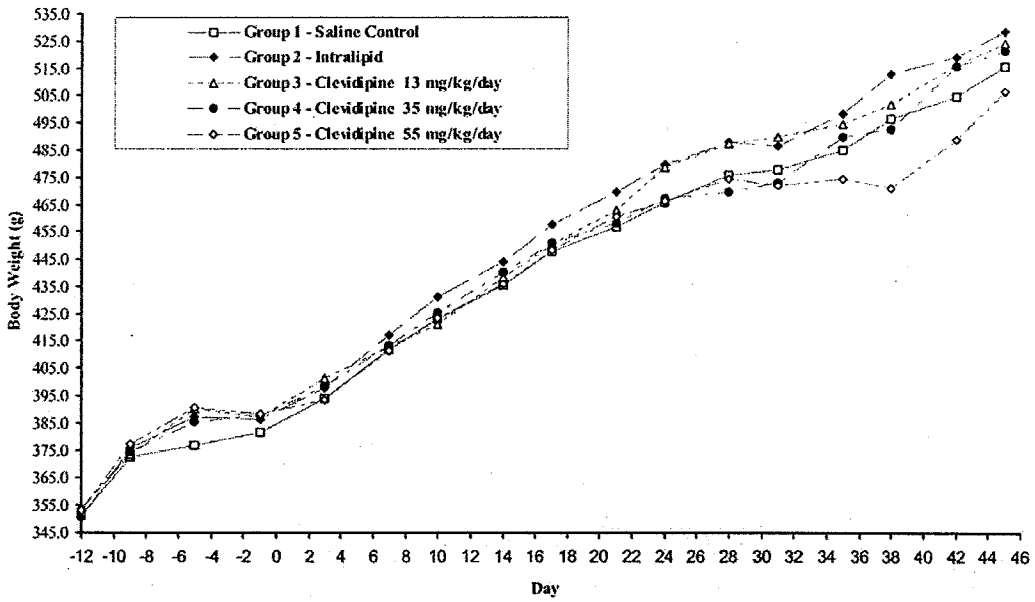
Group No. Identification	Dose Level (mg/kg/day)	Conc. (mg/mL)	Infusion Rate (mL/kg/hour)
1 Saline control	0	0	0.92
2 Intralipid (20%)	0	0	0.92
3 Clevidipine	13	0.6	0.92
4 Clevidipine	35	1.6	0.92
5 Clevidipine	55	2.5	0.92

a The females were untreated

Body weight

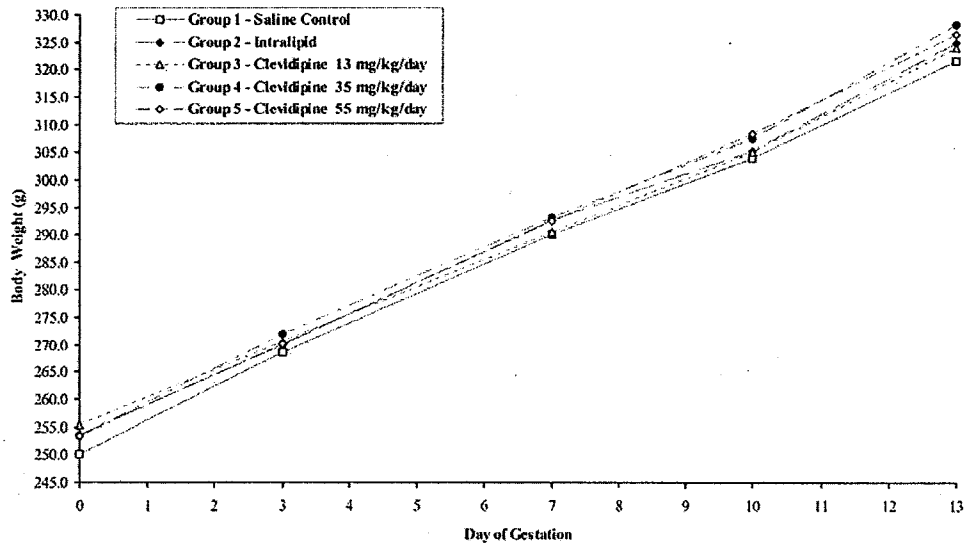
Body weight was affected at the HD in the last 10 days of the study. Was there a re-distribution of weight? The finding of prominent backbone increases with increasing dose of drug.

Figure 1 **Group Mean Body Weights - Males**



There is no apparent effect on body weight in the mated females.

Figure 2 **Group Mean Body Weights of Pregnant Females**



Summary of clinical findings of interest in males

	Dose group mg/kg				
	saline	Intralip	13	35	55
Abdominal distension	0	1	3	3	0
Backbone prominent	0	8	11	18	19
Cold to touch	2	2	7	9	8
dehydrated	0	4	6	7	6
Decreased feces output	0	3	4	3	0
Fur erected	0	2	3	4	8
Decreased muscle tone	3	8	7	16	17

Staining of the fur in the anogenital region, muzzle, abdomen and paws was also listed as was hunched posture.

Mean food consumption was decreased in the Intralipid and drug-treated males from day 3 of the study to day 24.

Absolute prostate weight was slightly decreased in the MD and HD groups (up to 7% compared to the saline control, n.s.). There was no discernible effect on absolute testes weight. Organs normalized to body weight did not show any discernible patterns.

There were no apparent drug-related effects on the testes. There were some reported effects on the lungs.

Table 9 Incidence of Necropsy Findings by Organ/Group

	MALE				
DOSE GROUP:	1	2	3	4	5
ORGAN/FINDING ANIMAL EXAMINED:	22	22	22	22	22
LIVER :					
- -Area pale- :	-	1	-	-	-
- -Area raised- :	-	1	-	-	-
- -Enlargement- :	-	-	2	2	-
- -Fissure- :	-	-	1	-	-
- -Foci pale- :	-	1	-	-	-
- -Mass- :	-	-	1	1	-
LUNG :					
- -Adhesion- :	-	-	1	1	-
- -Area dark- :	-	3	9	4	3
- -Area depressed- :	-	-	-	1	-
- -Area raised- :	-	-	-	1	1
- -Discoloration dark- :	-	-	1	2	1
- -Discoloration pale- :	-	-	1	5	4
- -Firm- :	-	-	1	-	-
- -Foci dark- :	1	6	4	10	12
- -Foci pale- :	-	-	-	2	2
- -Mottled- :	-	1	-	1	1
- -Nodule- :	-	-	1	-	-
- -Spongy- :	-	1	-	-	-
- -Uncollapsed- :	-	1	4	2	1
LYMPH NODE :					
- -Area dark- :	-	-	1	-	-
- -Cyst- :	-	1	-	-	-
- -Discoloration dark- :	-	2	3	1	2
- -Enlargement- :	-	5	6	12	8
- -Foci dark- :	-	1	-	1	-

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While there was no apparent treatment effect on fertility or conception rate, the saline control group had very low values for these parameters.

Group 1 - Saline Control
Group 2 - Intralipid
Group 3 - Clevidipine 13 mg/kg/day
Group 4 - Clevidipine 35 mg/kg/day
Group 5 - Clevidipine 55 mg/kg/day

Group	Number Placed for Mating: Males	Number Placed for Mating: Females	Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
1	22	22	21	1.9 (1.22) 21	17	95.5	77.3	81.0
2	21	21	21	3.2 (2.81) 21	19	100.0	90.5	90.5
3	18	18	17	3.0 (1.37) 17	17	94.4	94.4	100.0
4	17	17	17	2.4 (1.12) 17	17	100.0	100.0	100.0
5	21	21	21	2.8 (2.68) 21	20	100.0	95.2	95.2

There were no apparent drug-related effects on number of uterine implantation sites, number of live embryos and dead embryos, early resorptions, pre-implantation loss or post-implantation loss.

Overall Summary of Toxicology Findings

Single Dose Toxicity: Mice

Clevidipine was given via the tail vein to mice in a 1 minute duration injection. Doses used were 140, 180, 190, 220, 270, and 330 mg/kg. There was some inconsistency in the results. Animals at 180 mg/kg showed splayed gait and 1 animal showed dyspnea, cyanosis and convulsions followed by death. However, at 190 mg/kg , it was reported that there were no obvious signs. At ≥220 mg/kg, those who survived showed decreased motor activity, splayed gait, irregular and difficult breathing. While it was stated that 3 animals in the two highest dose groups died and another 3 required euthanasia, the distribution of the deaths/euthanasias is unclear.

Single Dose Toxicity: Rats

Clevidipine was given via the tail vein to rats in a 1 minute duration injection. Doses used were 23, 33, 93, 110, 140, and 160 mg/kg. Irregular breathing and decreased motor activity were reported for all doses for up to 45 minutes after dosing. At ≥93 mg/kg, ataxia was also reported. Mortality was seen ≥93 mg/kg, usually shortly after dosing, preceeded by dyspnea, cyanosis and sometimes convulsions.

The ataxia, dyspnea and convulsions are not unexpected sequelae to the exaggerated pharmacologic effects (hypotension and cerebral ischemia) produced by high doses of the drug.

Repeated Dose Toxicology

5 Day Intravenous Infusion (12 hours per day) dose range-findings study in the rat
Male and female rats received doses of 22, 66 and 198 mg/kg/day. Control animals received saline and 2 vehicle control groups received Intralipid 20%. No unscheduled mortality was reported for the main group. Mean rate of body weight gain was the same for the different groups within a given sex despite the decreased food intake with increasing dose of drug. Both HD males and HD females showed some decrease in platelets but this could be within the bounds of normal variability. Serum glucose was increased in MD and HD males (~15-20%) as was creatinine (0.4-0.6 mg/l). Neither glucose nor creatinine was perceptibly affected in females. Total bilirubin was approximately double in drug-treated rats compared to controls, but still within normal limits. Inconsistent increases in absolute and normalized liver and spleen weights were seen in both sexes. When the organ weight values from both sexes were combined, there was a dose-related increase in normalized lung weight.
Histologic findings included

Liver: single cell necrosis and increased mitotic activity (all groups)
Histiocytosis: all groups also. Possibly due to lipid vehicle
Lungs: moderate and multifocal hemorrhage (HD male)
Increased weight was attributed to incidental changes of hemorrhage and pneumonia
Adrenals: apparent dose-related increase in adrenal weight in drug-treated females

A maximally tolerated dose was identified as 137 mg/kg. The target organs of toxicity appeared to be liver, spleen, adrenal and possibly bone marrow depending upon the platelet decrease. How much of these apparent effects are due to the stress of the infusion process or the duration of infusion itself is not clear.

Complementary study: 7 day continuous IV (12 hours per day) infusion study in the rat
Male and female rats were given doses of 0, 6.6, 22, or 66 mg/kg/day. No treatment related signs were reported for this study. No treatment related signs were reported at necropsy.

4 Week Intravenous Infusion (12 hours per day) Toxicity Study in the Rat
Almost every animal in this study was ill with systemic sepsis.

96008 One Month Continuous IV Infusion Toxicity Study in the Rat

Male and female rats were dosed continuously with clevidipine for 28 days. Doses used were 0(vehicle), 23, 39 and 66 mg/kg/day.

Food consumption was decreased in all groups except the saline control. This might have been due to the caloric content of the Intralipid or due to the high incidence of keratitis. Body weight was apparently unaffected. Water consumption however, was increased in males (week 3, at 50 and 85 μmol/kg/day) and in females (week 3, 85 and 145 μmol/kg/day) and was also seen in females in the recovery phase.

There were no effects of toxicological significance in the clinical chemistry. In the hematology, there was a decrease in platelets of up to 20% associated with the vehicle. A regenerative anemia was apparent in the females at the end of the dosing phase but not the recovery stage. The sponsor did not mention the effects on the weight of liver, thyroid or testes in the textual summary.

organ	Dose group (μmol/kg/day)					Recovery dose	
	0(n=8)	0(n=8)	50(n=8)	85(n=10)	145	0(n=2)	145
Absolute organ weight							
Testes	3.525± 0.244	3.519± 0.229	3.288*± 0.158	3.144± 0.741	ND	2.400**± 0.735	
Prostate	0.665± 0.158	0.552± 0.192	0.653± 0.212	0.657± 0.255		0.792± 0.260	
liver	16.31± 2.70	16.25± 2.08	15.83± 1.59	17.06± 1.92		20.18± 3.77	
Left thyroid	16.2± 4.8	14.2± 2.2	14.3± 3.6	15.6± 3.1		21.1± 11.7	
Normalized organ weight							
testes	0.759± 0.069	0.747± 0.041	0.690± 0.064	0.660± 0.178		0.467**± 0.143	

As seen in other studies, liver and adrenal weight was affected in the females. It appears that a large part or all of this effect is due to the vehicle.

organ	Dose group (µmol/kg/day)				
	0(n=9)	0(n=10)	50(9)	85(n=10)	145(n=9)
Absolute organ weight					
adrenal	76.756± 10.029	108.220* ±39.456	86.089± 19.969	90.010* ±12.352	109.978**
liver	11.24±1.2	13.25**± 1.57	12.04± 0.89	12.54± 1.63	14.89**± 2.48

It was unexplained in the report why so many animals were available for organ weight determination, but so few for histopathological examination, as evidenced by the table of myocardial findings included in the review.

SE10192/Intralipid: One month continuous intravenous infusion investigative toxicity study in the rat

This study was to investigate the suitability of Intralipid as a vehicle for the rat. Three groups of 3 female Sprague Dawley rats were dosed with — 20% Intralipid at either 0.92 or 1.27 ml/kg/hour for 28 days. Two out of 3 females receiving 20% Intralipid at 1.27 ml/kg/hour were euthanized due to poor condition. The single male receiving the same concentration and rate of Intralipid was also euthanized due to poor condition but for some reason this was considered unrelated to treatment. One out of 3 female rats infused with — Intralipid at 0.92 ml/kg/hour showed signs of staining and hindlimb weakness. The clinical chemistry findings in this study appeared to be consistent with the infusion of a lipid rich material. The sponsor concluded that either — 20% Intralipid infused at 0.92 ml/kg/hour was tolerable for a 1 month infusion. This study would have been more valuable if conducted prior to the 1 month drug infusion study.

4-week intravenous infusion (12 hours per day) study in the Beagle dog

This was reviewed in the original submission by Preet Gil-Kumar and was not reviewed again here. The major findings from this study will be noted here. There were 3 dogs/sex/group given 0(control), vehicle(group2), 6.8, 16, 32 and 66 mg/kg/day clevidipine.

Animals from the vehicle, LD, MD and HD groups showed decreased activity, stiffness, weakness and subdued behavior. This was attributed to vehicle. In males the vehicle was associated with decreased rate of gain (veh, LD, ID, ID) and weight loss in the HD group. In females, weight loss was seen in all groups receiving the vehicle. In both sexes vehicle was also associated with decreased rbc count and decreased platelet count (approximately 80%). Decreased serum calcium and phosphorus were noted with the vehicle and were exacerbated in the MD and HD groups.

Increased heart rate occurred in all drug-treated groups of both sexes. Decreased PR and QT were associated with the positive chronotropic effect.

Mean testis weight of the drug-treated dogs was lower than both control and vehicle groups, consistent with findings from one of the rat toxicology studies. The adrenal weight of the HD f was increased, also consistent with the rat studies. Histologically this change corresponded to cortical hyperplasia of the adrenal zona glomerulosa. Something apparently unique to the dog study was ascending pyelonephritis and/or sometimes hemorrhages in the urinary bladder or cystitis, unusually severe mixed cell infiltration in the liver (all groups but more severe in groups 2-6) and lymphoid atrophy in the thymus (groups 2,4,5 and 6 males and groups 2-6 females). Samples for urinalysis were collected by catheterization. The damage and infections in the bladder may well have been secondary to less than optimal catheterization techniques. The finding was reported for 1 HD m, 1 vehicle f and several females from groups 5 and 6. The pathologist's report states that almost all males and females in groups 2-6 had acute interstitial nephritis located in the papilla or in the inner medulla of the kidneys. Two females from group 5 had an area of renal necrosis associated with the nephritis.

The effect on the testes was a point of discussion at a meeting with the sponsor on July28, 2004. Shown below is a paragraph from the minutes taken by C. Resnick, Ph.D.

> ***3. Clevidipine-related reduction in weight of testes of dogs in a one month repeat-dose study.***
>
> Mean absolute and relative testis weights were dose-relatedly lower for clevidipine treated groups than for either Intralipid or Saline Control groups. In this study, 4 dose levels of clevidipine were evaluated by continuous infusion (6.8, 16, 32 and 66 mg/kg/day). There were 3 males and 3 females in each control and clevidipine treatment group. Absolute testes weights 53-62% lower than the lowest control value (a saline control animal) were recorded in one dog dosed with 6.8 mg/kg/day (1.53g), one dog dosed with 32 mg/kg/day (1.69g) and two dogs dosed with 66 mg/kg/day (1.39g and 1.75g). Relative weights 34-49% lower than the lowest control value (same saline control animal as above) were recorded in these same clevipine-treated animals (one dog dosed with 6.8 mg/kg/day (0.023),one dog dosed with 32 mg/kg/day (0.030) and two dogs dosed with 66 mg/kg/day (0.024 and 0.031). The sponsor indicates that the group differences in absolute weights are not statistically significant and that the group differences in relative weights are statistically significant only for the highest dosage group (assume that the sponsor is referring to the comparison of high dose vs saline control). Furthermore, the sponsor notes the absence of macroscopic or microscopic changes in the testis other than indications of immaturity. (Dogs were about 20 weeks old at the initiation of treatment and, according to the sponsor, development of testis, epididymis and prostate is marked in dogs between 20 and 32 weeks of age.) Finally, the sponsor notes that at 66 mg/kg/day, the only dose at which they appear to acknowledge an effect of clevidipine, there is a significant margin compared to the anticipated dosing in humans (8 mcg/kg/min for up to 2 hours; total daily dose of 960mcg). There was no attempt to determine reversibility of the effect.

Reproductive Toxicology

August 30 , 2006, the sponsor submitted several studies to try to address the concerns about the reproductive toxicity issues (sequence number 059, annual report). These studies were: "A continuous intravenous infusion toxicity study of clevidipine in nonpregnant New Zealand White rabbits" , " A continuous infusion teratology study of clevidipine in the rabbit", "A continuous intravenous infusion pre and postnatal study of clevidipine in the rat", and "A 14-day intravenous infusion toxicity study of clevidipine in the male Beagle dog with a 28-day recovery period. "

900624"A continuous intravenous infusion toxicity study of clevidipine in nonpregnant New Zealand White rabbits"

The point of this study was to evaluate the feasibility of dosing Intralipid 20% as a vehicle and clevidipine formulated in Intralipid to rabbits for the definitive teratology study. The text reports that in a previous dose-ranging study (Astra 96160) and a teratology study (Astra 97010) performed using Dutch rabbits, a "significant" number of rabbits died, were euthanized or aborted their pregnancies at all doses of clevidipine and in the Intralipid control group. The high dose infusion rate was 0.92 ml/kg/hour administered for 24 hours. The current study duplicated the doses of Intralipid and clevidipine used previously.

Summary of doses used.

Group	Dose (mg/kg/day)	Infusion rate (ml/kg/hour)	# of females
Intralipid 20%	0	0.92	3
clevidipine	55	0.92	2

In this study, non-pregnant NZW rabbits received either intravenous Intralipid or intravenous clevidipine formulated in Intralipid for 14 days. There was no unscheduled mortality. No gross lesions were reported. We do not have an untreated control group or a saline infusion group to compare weight gain and food consumption.

900591 A continuous intravenous infusion teratology study of clevidipine in the rabbit

NZW rabbits were dosed according to the table below.
Summary of dosing

	Dose mg/kg/day	Conc Mg/ml	Infusion rate Ml/kg/h	# of females
Saline control	0	0	0.92	25
Intralipid control	0	0	0.92	25
Clevidipine	13	0.59	0.92	25
Clevidipine	35	1.59	0.92	25
clevidipine	55	2.49	0.92	25

There was no clear trend of increased anomalies with treatment. No maternal NOAEL was identified. The sponsor concluded a developmental NOAEL of 35 mg/kg/day. Dystocia and delayed parturition were seen. These effects are consistent with effects identified with other calcium channel blockers due to the role of calcium in muscle contraction.

900510 A continous infusion pre and postnatal study of clevidipine in the rat

The sponsor tested the effect of clevidipine upon gestation, parturition and lactation by administering the drug to the dams from Day 6 of gestation to the morning of post-partum day 4. The doses used are summarized in the table below.

Summary of study design

group	Dose (mg/kg/day)	Concentration(mg/ml)	# of animals(dams)
Saline control	0	0	24
Intralipid control	0	0	24
clevidipine	13	0.6	24
clevidipine	35	1.6	24
clevidipine	55	2.5	24

Maternal performance and the F1 viability index for PN 4 were both affected.

Sponsor's summary of maternal performance

dose	Gestation index(%)	Gestation length (days)	Live litter size	Avg # dead pups /litter	Live birth index (%)
Saline control	100.0	21.3	13.2	0.05	93.6
Intralipid	79.2	21.4	12.8	0.68	89.2
Clevidipine 13 mg/kg/day	69.6#	21.7#	10.5#,@	0.94	75.1#
Clevidipine 35 mg/kg/day	63.6#	22.1#,@	5.3#,@	0.93	39.6#,@
Clevidipine 55 mg/kg/day	9.1#,@	22.6#,@	2.5#,@	1.50	22.2#,@
HCD	95.0-100.0	21.6-22.0	13.0-15.6	0.0-0.47	89.0-94.9

HCD= historical control data
#= significantly different from saline control group
@=significantly different from the Intralipid control group

Day 4 viability index summary

	saline	intralipid	Clevidipine 13 mg/kg	Clevidipine 35 mg/kg
Viability index day 4	97.7±3.50 N=22	90.0±25.09 N=19	86.5±30.60 N=14	78.3±40.21 N=6

There was an apparent trend in the reproductive performance of the F1 animals. Mating index, fertility index and conception rate decreased in adults who had early exposure to clevidipine.

Table 37 **Group Mean Parental Performance**

F1 Generation - Adults

Group 1 - Saline Control
Group 2 - Intralipid Control
Group 3 - Clevidipine 13 mg/kg/day
Group 4 - Clevidipine 35 mg/kg/day

Group	Number Placed for Mating: Males	Number Placed for Mating: Females	Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
1*&	22	22	22	4.8 3.01 (N: 22)	22	100.0	100.0	100.0
2 *&	17 a	18	18	4.2 2.88 (N: 15)	18	100.0	100.0	100.0
3 &&	25 b	26	26	3.6 2.31 (N: 25)	24	100.0	96.0	92.3
4 &&	8 c	10	9	4.1 2.85 (N: 8)	8	87.5	75.0	88.9

a = Male 226 paired with females 270 and 280
b = Male 303 paired with females 356 and 366
c = Male 404 paired with females 455 and 459 and male 409 paired with females 456 and 458
Significantly different from control group (group 1) value: X - P ≤ 0.05 Y - P ≤ 0.01 Z - P ≤ 0.001 (Wilcoxon - day to mating only)
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)
Significantly different from control group (group 2) value: x - P ≤ 0.05 y - P ≤ 0.01 z - P ≤ 0.001 (Wilcoxon - day to mating only)
Significantly different from control group (group 2) value: + - P ≤ 0.05 ++ - P ≤ 0.01 +++ - P ≤ 0.001 (Fisher's)
*&: Subset 2
&&: Subset 1 and 2

Given the small number of animals, it's difficult to say how real the effects are in either the parental or maternal summaries. The primary effect noted in maternal effects is a decreased pregnancy rate.

Summary of maternal effects

group	# mated females	# pregnant females	Preg rate (%)	Gestation index(%)
Saline	22	22	100	100
Intralipid	18	18	100	100
Clev 13mg	26	24	92	96
Clev 35mg	9	8	89	100

Viability and weight were apparently unaffected in the F2 generation.
There is no record of any examination of the male reproductive tract of any generation beyond gross observation.
Continuous intravenous administration of clevidipine in a lipid emulsion at doses of 13, 35 and 55 mg/kg/day caused dose-related increases in F0 maternal mortality, mostly in late gestation. The increased mortality was primarily related to complications with parturition, most likely due to interference with uterine contractions, an effect reported with other calcium channel blockers such as nicardipine. In this study, no maternal NOAEL was identified. Dose-related gross pathology was associated with the infusion sites, lung, adrenals, liver and spleen. The sponsor attributed these findings to the drug.

Evaluation of the F1 generation was somewhat compromised by how few members of the F1 generation survived. F1 viability (PN days 0-4) showed a dose-related decrease and an effect from the lipid vehicle.

There were slight decreases in the time for F1 drug-treated pups to reach the day of tooth eruption and eye opening. F1 females showed a slight increase to the time of vaginal opening. The significance, if any, of these observations is unclear.

Fertility in the F1 generation was apparently decreased in those pups who had received early exposure to clevidipine. Unfortunately there is no record of any histologic examination of the male reproductive tract nor assessment of sperm characteristics.

500943 A 14-day intravenous infusion toxicity study of clevidipine in the male Beagle dog with a 28-day recovery period.

The study design is summarized in the sponsor's table below.

Group	Dose (mg/kg/day)	Conc (mg/ml)	Dose rate (ml/kg/hr)	Animal number	
				main	recovery
Saline	0	0	1.84	4	2
20% lipid emulsion	0	0	1.84	4	2
clevidipine	6.8	3.0	0.19	4	2
clevidipine	16	3.0	0.44	4	2
clevidipine	66	3.0	1.84	4	2

In a previously conducted 28 day study testing the same dose levels proposed for the current study, dose-related decreases in absolute and relative testes weights were observed. No histopathology observations were noted in the testes other than those usually associated with immature dogs. This study was conducted in mature (13 months to 5 years) male Beagles to explore a possible testicular effect.

Unfortunately, the sponsor chose to use ejaculated samples for the assessment. The study was made almost un-interpretable due to the variability of measurements. The pathologist noted that:

> Male reproductive assessments were difficult to interpret due to individual variability, which was not influenced by the treatment. In many animals, regardless of their dose group, the collection was unsuccessful at many occasions or the ejaculated volume was too small to be analyzed or interpreted. In addition, many samples had no spermatozoa. However, in animals/sampling occasions where sufficient ejaculated volume/spermatozoa counts were produced, the administration of the vehicle with or without clevidipine did not have any effect on the sperm motility, spermatozoa counts or on the spermatozoa morphology.

There were unsuccessful semen collection attempts on the dogs who later were determined to have hypo/aspermatogenesis. The sperm count, motility and morphology were determined only on the ejaculated samples. Post-mortem, only histopathology was assessed. A determination of spermatozoa/unit weight would have been helpful. Also, the methods and protocol state that spermatogenic staging had been performed. I did not find any summary of findings nor did I find any reference to the findings.

The pathologist also adds a comment that the hypo/aspermatogenesis seen in 4 dogs (1LD, 1 MD and 2 HD) was suggestive of immaturity. They cite a report stating that up to 6% of Beagles 2.5-7.5 years show incomplete spermatogenesis (Rehm, S. Spontaneous testicular lesions in purpose-bred Beagle dogs. Toxicol Pathol 28, 782-787, 2000.

I would be more inclined to accept the pathologist's proposal that the hypo/aspermatogenesis was a background lesion if:

1. the statistical probability of that 6% incidence occurring 4 times in 3 drug-treated groups was higher
2. if the cases of hypo/aspermatogenesis occurred also in the control groups
3. if clevidipine was not a calcium channel blocker, drugs which appear to have a class effect of interfering with spermatozoa via the calcium ion channels.
4. if there was not a decrease in testicular weight in drug-treated dogs.

It is also problematic that this study was only 2 weeks of treatment. In that time the only obvious effect in ejaculated sperm will be from a direct toxic effect on epididymal sperm. However, I searched MicroMedex to see how clevidipine compares to approved calcium channel blockers in this respect. The 3 page summary of that search is included as Appendix 2. There does appear to be a class effect of calcium channel blockers on several aspects of fertility. Calcium plays important roles in sperm activation during fertilization. Calcium is also involved in muscle contraction and so has been associated with delayed parturition and dystocia. In fact verapamil has been used to stop human pre-term labor. A small number of cases show verapamil and several other calcium channel blockers to be associated with reversible male infertility although there is some debate whether other factors may be involved.

In a meeting with the sponsor in 2007, the [redacted] was discussed.

2.6.6.9 Discussion and Conclusions

Clevidipine is a dihydropyridine calcium channel blocker prepared as a racemic mixture of the R- and S- enantiomers. In vitro, the pharmacodynamic properties of the enantiomers appear to be equal. The major metabolite, M1 (H152/81) appears 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 of clevidipine is greatest in anesthetized SHR rats, followed by conscious SHR and lowest in normotensive rats.

Assessing the general toxicology of clevidipine is somewhat confounded by the vehicle Intralipid20% that was used for most studies. The vehicle caused clinical chemistry and histological effects by its nature as a lipid dense material. The vehicle also appeared to exacerbate some of the effects of clevidipine. Another confounding item is the manner in which the toxicology studies were conducted. The animal husbandry techniques can be surmised to have been sub-optimal in a number of studies as witnessed by almost universal sepsis, catheter closures, keratitis, poor overall survival and precipitation of drug material in the catheters. Sometimes the evaluation of the methodology was done after the "definitive" study.

The two major non-clinical issues in this NDA are reproductive effects and the genotoxicity. The reproductive toxicity includes prolonged parturition, dystocia, impaired male fertility and altered in utero development of the kidneys. Similar effects have been reported for approved calcium channel blockers. There are also some case reports of impaired male fertility that have reversed when use of a calcium channel blocker ceased. What appears to be an unusual effect of clevidipine is pseudopregnancy in rats and estrus cycles of unusual length. The details of the reproductive tracts of the female dogs was not readily apparent in the study reports and even if those details were available, it is not certain that they would be of use. The female dog is suboptimal for answering these reproductive issues in light of mono-seasonal estrous and the fact that pseudopregnancy is very common.

A serious concern is the production of formaldehyde as one of the metabolites of clevidipine.

ACGIH lists formaldehyde as a suspected human carcinogen (A2), EPA calls formaldehyde a probable human carcinogen (B1). The Gene-Tox database lists the following for formaldehyde:

Human cells, Sister-chromatid exchange in vitro: positive with dose response

Human lymphocytes, SCE in vitro: positive with dose-response
Nonhuman cells, SCE in vitro: positive with dose-response
Other assays were listed as pre-1980

Formaldehyde is an endogenous metabolic product of N-, O- and S-demethylation reactions within cells. With its oxidation product, formate, it is an intermediate in the one-carbon pool, used for the biosynthesis of purines, thymidine and certain amino acids. As such, formaldehyde is present within all tissues, cells and bodily fluids. Circulating concentrations of approximately 2.6μg/g blood (70μM) have been reported for people not exposed to exogenous formaldehyde(McGregor, 2006). Another source lists endogenous formaldehyde in the blood of rats, humans and monkeys as approximately 0.1 mM(Heck and Cassanova, 2004).

Metabolism of endogenous formaldehyde is reported to occur by spontaneous combination with reduced glutathione to form S-hydroxymethylglutathione, the substrate for alcohol dehydrogenase 3 (ADH3, glutathione-dependent formaldehyde dehydrogenase). This is further metabolized to formic acid and reduced glutathione by S-formylglutathione hydrolase.

While formaldehyde has been extensively investigated for its potential role in leukemia, recent work has also begun to explore the possible connection with Alzheimer's disease. Aldehydes, endogenous or not, are capable of inducing protein cross-linking. The protein cross-linking properties make formaldehyde useful for tissue preservation.

It is also interesting to note that immediate effects of formaldehyde exposure include decreased blood pressure, heart rate, peripheral resistance and cardiac output. These cardiovascular effects were reported in rats following infusion of 0.01 ml formalin (formaldehyde at 0.12 mmol/kg/min). Abnormalities in male fertility have also been shown to result due to formaldehyde exposure of 10 mg/kg/day for 30 days(Meditext®).

Inhalation of formaldehyde vapors at ≥2-5 ppm causes irritation of the pharynx and lungs and can progress to bronchitis, pulmonary edema and pneumonia. Sensitive individuals may have reactions at concentrations as low as 0.1 ppm(Meditext ®).

There is another approved drug where formaldehyde is a metabolite.

1. Uroqid®-Acid No.2 (methenamine mandelate/sodium acid phosphate. Each tablet contains 500 mg methenamine mandelate and 500 mg sodium acid monophosphate. This is given 2-4 tablets daily for suppression or elimination of bacteriuria associated with chronic and recurrent infections of the urinary tract. The following is directly from the product label:

For the suppression or elimination of bacteriuria associated with chronic and recurrent infections of the urinary tract, including pyelitis, pyelonephritis, cystitis, and infected residual urine accompanying neurogenic bladder. ***When used as recommended, UROQID-Acid® No.2 is particularly suitable for long-term therapy because of its relative safety and because resistance to the nonspecific bactericidal action of formaldehyde*** does not develop. Pathogens resistant to other antibacterial agents may respond because of the nonspecific effect of **formaldehyde** formed in an acid urine. [emphasis added by reviewer]

1. PROSED/DS® : methenamine (81.6 mg), phenyl salicylate, methene blue, benzoic acid and hyoscyamine sulfate. The following information is directly from the product label.

   "PROSED/DS® is indicated for the relief of discomfort of the lower urinary tract caused by hypermotility resulting from inflammation or diagnostic procedures and in the treatment of cystitis, urehtritis and trigonitis when caused by organisms which maintain or produce an acid urine and are susceptible to formaldehyde."

Goodman and Gilman, 9th edition, provides more information regarding methenamine. Methenamine decomposes in water to release formaldehyde. At pH 7.4 almost no decomposition occurs. At pH 6, 6% of the theoretical maximum is produced and 20% at pH 5. Thus acidification of urine promotes formaldehyde-dependent antibacterial action. The reaction is slow, requiring 3 hours to reach 90% completion. Two grams of methenamine yields formaldehyde at 18-60 mg/ml urine. Recommended daily levels are 1-2 grams divided into 4 doses. An immediately obvious difference between methenamine and clevidipine is that methenamine's formaldehyde production is restricted to the urinary tract and has significantly less tissue-residence time than clevidipine.

NIOSH lists the TWA(8 hours a day for a 40 hour work week) for formaldehyde as 0.016 ppm or for a 15 minute interval, 0.1 ppm.

The proposed dose range is:

2 mg/hr up to 32 mg/hr
0.56μg/kg/min up to 8.9μg/kg/min for a 60 kg patient

Worst-case scenario of one molecule of clevidipine generating 1 molecule of formaldehyde

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

Conversion factor used: 1ppm=1mg/kg=1μg/g

The sponsor made the following calculations for potential formaldehyde exposure in humans:

Potential for Formaldehyde Formation in Humans:

The potential for formaldehyde formation in humans was assessed based on clevidipine's metabolism. 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. Formaldehyde concentration is expected to be highest when a high infusion rate of clevidipine is administered (8 µg/kg/min, 17.53 nmol/kg/min). At this highest formation rate of formaldehyde, the highest steady-state formaldehyde concentration = formaldehyde formation rate/formaldehyde clearance = 17.53/46.2 = 0.379 nmol/mL. The baseline formaldehyde concentration reported by Heck et al 1983 (reference contained in the original NDA) was 2.61 µg/g (86.9 nmol/mL). Therefore, given a maximum clevidipine dose of 8 µg/kg/min (or ~40 mg/h, which exceeds the maximum proposed does of 32 mg/h), the expected formaldehyde concentration (0.379 nmol/mL) is at least 200 times lower than the baseline endogenous concentration (86.9 nmol/mL). This indicates that the maximum anticipated formaldehyde levels resulting from clevidipine administration are not distinguishable from endogenous formaldehyde levels.

The baseline formaldehyde concentration cited above were obtained from Fischer rats exposed to 15 ppm formaldehyde for 6 hour a day for 9 days then on the 10th day rats were exposed in a head-only chamber to 14.9 ppm $^{14}CH_2O$ for 6 hours. A second group of rats with no prior exposure to formaldehyde were also exposed to the $^{14}CH_2O$ vapor. After reading the reference,it was clear that the sponsor had made an error in the citation. However, the author of the manuscript did conclude that

> The disposition and pharmacokinetic studies indicate that inhaled CH_2O is extensively metabolized. The high level of residual radioactivity in tissues collected 70 h post exposure and the approximately equivalent amount that is oxidized to $^{14}CO_2$ during the same time period support this concept. It is likely that folic acid plays an important, perhaps a preeminent, role in this incorporation (6). However, the possibility that $^{14}CH_2O$ may form covalent adducts in vivo (15) or that $^{14}CO_2$ may itself be incorporated via carboxylation reactions cannot be excluded. The metabolism of inhaled $^{14}CH_2O$ appears to be similar to that which occurs following other routes of administration (1-3).

(Heck H., TY Chin and MC Schmitz :"Distribution of [^{14}C]formaldehyde in rats after inhalation exposure" in Formaldehyde Toxicity ed JE Gibson. McGraw-Hill International Book Company, Washington. 1983.) A second (uncited) publication was examined and actually contained the cited average concentrations of CH_2O of µg/g blood of 2.61±0.14 in humans (Heck et al. "Formaldehyde concentrations in the blood of humans and Fischer-344 rats exposed to CH_2O under controlled conditions." Am Ind Hyg Assoc, J 46(1):1-3(1985).)

APPEARS THIS WAY
ON ORIGINAL

The sponsor was asked to provide a radio-label distribution study to see the extent of the distribution and if the drug-associated radioactivity persists. The drug is widely and

rapidly distributed and persists in some tissues for up to 672 hours (the last point of sampling). As the parent drug was labeled, the ^{14}C would go with the formaldehyde moiety. We do not know if the formaldehyde is persisting in tissues as formaldehyde (cross-linking proteins) or if it persisting in the one-C pool or incorporated into protein or genetic material. The sponsor was unable to provide any measured levels of formaldehyde. All numbers were based upon theoretical expectations of amount produced and projected clearance. I have forwarded the sponsor's calculations to Lydia Velasquez, Ph.D., the biopharmaceutics reviewer and asked if she has any information to apply to this question.

CDER's Genetic Toxicology Committee was given the genetic toxicology background for clevidipine as well as the human exposure calculations. The following table was included in the consult request:

Clevidipine Genotoxicity Summary

Report	Assay	Concentrations/strains	Results
7616-100	Ames	TA98, TA100, TA1535,TA1537, E.Coli WP2*uvr*A ±S9 1st assay: 10, 33, 100, 333, 1000, 2000, 5000µg/plate 2nd: 1500, 2500, 3000, 4000µg/plate	Positive results in TA98 and TA100 +S9
7616-101	Ame	TA98 and TA100, ±S9 and ±formaldehyde dehydrogenase(FDH) 33.3, 100, 1000, 1500, 2000, 2500, 3000, 4000, 5000µg/plate	Positive result in TA100 and trend in TA98 +S9
961056*	Ames	TA1535, TA1537, TA98, TA100, TA102, E.coli WP2 *trp uvr*A, ±S9, ±FDH 28, 50, 89, 158, 281, 500, 889, 1581,2812,5000µg/plate Formaldehyde included as +control	Positive results TA100, TA98, TA102 +S9
T2833	Ames (Astra)	TA1535, TA100, TA1538, TA98, TA1537 ±S9 145, 485, 1450, 4850,14500µg/plate	Negative (inconsistent weak positive in TA100 +S9)
T2886	Mouse lymphoma (Astra)	Formaldehyde tested	positive
T2891	Mouse lymphoma (Astra)	Clevidipine ±FDH ±S9; formaldehyde as positive control	Positive ±S9 (greater mean mutation frequency than the positive control +S9)

Clevidipine Genetic Toxicology Summary Continued

Report	Assay	Concentrations/strains	Results
T3363	Human lymphocyte transformation test (in vitro)(Astra)	-S9: 0.146, 0.073, 0.0365 g/l No representation of metabolites in the test system	No significant increase in DNA synthesis
T3376	Chromosome aberration in vitro		Increased mitotic index -S9: statistically significant increase in the frequency of aberrations at highest concentration +S9: dose-related increased frequency of aberrations
T3376	Chromosome aberration in vitro	Human lymphocytes Repeated with FDH	-S9: at HD there was ↑in abnormal metaphases and ↑mitoses/1000 cells Adding FDH ↓ abnormal metaphases but not the ↑mitoses/1000 cells +S9: dose-related ↑in abnormal metaphases In the presence of FDH there was no increase in abnormal metaphases.
T3367	Mouse micronucleus (Astra)	Single IV doses of 8.2, 41 and 82 mg/kg Bone marrow	negative

- The study report notes that the tester strains showed sensitivity to formaldehyde. The response to formaldehyde and to the test article was generally decreased by the presence of FDH.

The Genetic Toxicology Committee was given 2 questions:

2. What level of concern is there for the persistence of drug-associated radioactivity ?
3. What level of concern is there for metabolism of the drug to formaldehyde?

The Committee's responses were not helpful and are included in Appendix 1.

OVERALL CONCLUSION D. S AND RECOMMENDATIONS

Conclusions: The drug is approvable pending resolution of the genetic toxicology issue.

Unresolved toxicology issues (if any): The genetic toxicology is unresolved.

Recommendations: see Executive Summary

Suggested labeling: see Executive Summary

Reviewer: NDA No.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

APPENDIX 1

----- Original Message -----
From: Greg Williams <greg.williams@THEMEDCO.com>
To: Hausner, Elizabeth A
Cc: Defelice, Albert F; Karkowsky, Abraham M; Hinton, Denise; Lori Lucas <lori.lucas@themedco.com>
Sent: Fri Dec 21 13:55:20 2007
Subject: RE: NDA 22-156 Clevidine

Dear Elizabeth,

As you know, clevidipine is a dihydropyridine calcium channel blocker. Given the level of structure / efficacy, specificity and safety relationship knowledge related to this drug class and clevidipine in particular, this level of screening was not thought to be warranted. With this in mind, no 'panlab screening' testing was performed.

We would be glad to discuss any further questions that you may have on this matter during the week of January 7th and ask that you please let me know if you think that this would be helpful.

In the mean time, please have a terrific holiday season.

Thank you,
Greg

Gregory C. Williams, Ph.D.
Vice President , Regulatory Affairs and Program Management
The Medicines Company
8 Campus Drive
Parsippany, New Jersey 07054
973-647-6010

From: Greg Williams
Sent: Wednesday, December 19, 2007 8:49 AM
To: 'Hausner, Elizabeth A'; 'denise.hinton@fda.hhs.gov'
Cc: 'albert.defelice@fda.hhs.gov'; 'abraham.karkowsky@fda.hhs.gov'
Subject: RE: NDA 22-156 Clevidine

Hi Elizabeth,

Thank you for your clarification. I will look into this matter and get back to you before the end of the week.

Regards,

Greg

From: Hausner, Elizabeth A [mailto:elizabeth.hausner@fda.hhs.gov]
Sent: Wednesday, December 19, 2007 8:15 AM
To: Hinton, Denise; Greg Williams
Cc: Defelice, Albert F; Karkowsky, Abraham M
Subject: RE: NDA 22-156 Clevidine

Greg,
what I am referring to is a screening assay in which the drug is tested against approximately 100 different receptors, such as the receptors of the neural system, steroidal receptors, the different receptors of the vascular system, etc. I've already seen the reports that you referenced and need the bigger picture.
Thank you,
Elizabeth

Elizabeth Hausner DVM, DABT,DABVT
Senior Pharmacologist
Division of Cardio-Renal Drug Products
USFDA/CDER
301-796-1084 telephone

From: Hinton, Denise
Sent: Tuesday, December 18, 2007 4:42 PM
To: Hausner, Elizabeth A
Subject: FW: NDA 22-156 Clevidine

From: Greg Williams [mailto:greg.williams@THEMEDCO.com]
Sent: Tuesday, December 18, 2007 4:09 PM
To: Hinton, Denise
Cc: Lori Lucas
Subject: FW: NDA 22-156 Clevidine

Hi Denise,

In response to your below question, we believe that you are referring to the receptor binding report(s) which describe clevidipine's mechanism of action.

The two most relevant receptor binding studies are Study Report 2220-870-00 (this is an in vitro study in cortical neuronal cells showing that clevidipine is a calcium channel blocker preventing calcium influx in depolarized neurons) and Study Report 2220-0854-00 (this is an in vitro study in isolated rabbit myocytes showing the effect of clevidipine on the L-type calcium channel current). Collectively, these two

studies demonstrate that clevidipine inhibits cellular calcium influx and blocks the L-type calcium channel. The locations of these reports in the original NDA are contained in the following table:

Studies which demonstrate that the mechanism of action of clevidipine is through the inhibition of transmembrane calcium transport via effect on the L-type calcium channel

Study Report Title
Study Report Number
NDA Location

Effect of the Short-Acting Dihydropyridine H 324/38 on Potassium-Induced Calcium Influx in Primary Cortical Neuronal Cell Cultures from Neonatal Rats.

2220-870-00

Module 4/ 4.2.1.1.1 Report 2220-870-00

Effect of H 324/38 on the L-Type Calcium Current in Rabbit Ventricular Myocytes: A Comparison with Nicardipine

2220-0854-00

Module 4/ 4.2.1.1.9 Report 2220-854-00

For your convenience, these reports areattached to this email as PDF files.

These studies are also discussed inSection 2.6.2.2.1 of the original NDA (Inhibition of transmembrane calcium transport via effects on the L-type calcium channel in vitro).

Please let me know if we may be of further assistance.

Thank you,

Greg

APPEARS THIS WAY
ON ORIGINAL

From: Hinton, Denise [mailto:denise.hinton@fda.hhs.gov]
Sent: Tuesday, December 18, 2007 10:07 AM
To: Greg Williams
Subject: NDA 22-156 Clevidine

Hi Greg,

Would you please provide the number for the study report that contains the receptor binding study?

Thank you,

Denise

APPENDIX 2

EMAIL SERIES 1

Elizabeth,
It seems there may be two issues here, one formaldehyde and the other persistence of the drug for a very protracted period. If the label is in that part of the molecule that ends up as formaldehyde, it may only be a single issue. The persistence may reflect covalent binding. Will all the studies be done in patients? Again, since this is a 3 day exposure that is likely only to take place once in a lifetime, the risks are likely to be small.

David Jacobson-Kram, Ph.D., D.A.B.T.
Office of New Drugs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
Phone: 301-796-0175
Fax: 301-796-9856
email: david.jacobsonkram@fda.hhs.gov

From: Hausner, Elizabeth A
Sent: Wednesday, November 21, 2007 9:42 AM
To: Jacobson-Kram, David; Levy, Dan; Robison, Timothy W; Atrakchi, Aisar H; Elespuru, Rosalie K.; Heflich, Robert; Bigger, Anita; Ouyang, Yanli; Moore, Martha; Agarwal, Rajiv; Benz, Robert Daniel; De, Mamata; Jagannath, Devaraya R; McGovern, Timothy J; Nostrandt, Amy C; Sheu, Chingju W; Sotomayor, Rene E; Yao, Jiaqin
Subject: RE: CDER Genetic Toxicology Subcommittee Consult

My summary to the committee was obviously not clear and I apologize for the confusion. There is no planned radio-label study in humans. The radio-labeled data is from a distribution study in rats. The rat study showed rapid distribution of the metabolites (formaldehyde is not insignificant in quantity) and persistence of the drug-associated radioactivity out to the final point of sampling, which was 28 days after the single bolus dose. The drug especially seems to home for the brain, endocrine system, and reproductive tract. The proposed use for this drug would be for up to 72 hours of continuous infusion in humans. What is the level of concern given the known genetic toxicity profile of formaldehyde and the persistence in tissues ? There is also a recent body of research looking at the potential role of endogenous aldehydes in neuropsychiatric disorders such as Alzheimers disease (cross-linking proteins is what make formaldehyde so useful in histology).
Thank you all for your time and consideration in this.

From:	Jacobson-Kram, David
Sent:	Tuesday, November 20, 2007 2:54 PM
To:	Levy, Dan; Robison, Timothy W; Atrakchi, Aisar H; Elespuru, Rosalie K.; Heflich, Robert; Bigger, Anita; Ouyang, Yanli; Moore, Martha; Agarwal, Rajiv; Benz, Robert Daniel; De, Mamata; Jagannath, Devaraya R; McGovern, Timothy J; Nostrandt, Amy C; Sheu, Chingju W; Sotomayor, Rene E; Yao, Jiaqin
Cc:	Hausner, Elizabeth A
Subject:	RE: CDER Genetic Toxicology Subcommittee Consult

Radio labeled studies are done all the time in human volunteers. The radiation doses are negligible.

David Jacobson-Kram, Ph.D., D.A.B.T.
Office of New Drugs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
Phone: 301-796-0175
Fax: 301-796-9856
email: david.jacobsonkram@fda.hhs.gov

From: Levy, Dan
Sent: Tuesday, November 20, 2007 2:51 PM
To: Robison, Timothy W; Atrakchi, Aisar H; Elespuru, Rosalie K.; Jacobson-Kram, David; Heflich, Robert; Bigger, Anita; Ouyang, Yanli; Moore, Martha; Agarwal, Rajiv; Benz, Robert Daniel; De, Mamata; Jagannath, Devaraya R; McGovern, Timothy J; Nostrandt, Amy C; Sheu,

Chingju W; Sotomayor, Rene E; Yao, Jiaqin
Cc: Hausner, Elizabeth A
Subject: RE: CDER Genetic Toxicology Subcommittee Consult

Formaldehyde has been around for a long time. Long enough so that much of the relevant literature is in textbooks or pre-PubMed. It is a classic adduct-former and crosslinking agent. According to Singer and Grunburger (Molecular Biology of Mutagens & Carcinogens 1983 Plenum pp53-5) it is also reactive enough so that it is thought to react with proteins and other cellular components long before it gets to the nucleus or via reactive oxygen species generated by formaldehyde-induced catylase inhibition. These all suggest that it is easy to generate mechanisms of general or genetic toxicity at high levels of exposures that are not relevant to low level exposure.

My main concern here is the radio-labeling study. I am not enough of a chemist to guess at how this compound is metabolized. If the radiolabeled carbon is the one that is metabolized into formaldehyde I would frame the question this way: do you really want to be randomly alklylating macromolecules with C14 in normal volunteers? I am not familiar with how frequently this is done during drug development. If it is rare, I would not suggest that this might not be a good candidate to break that pattern. It is not clear to me what would be gained by the radiolabel study in humans.

Dan D. Levy, Ph.D.
Senior Microbiologist
Division of Dietary Supplement Programs
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740
301 436 2581 {tel.} {fax} 301 436 2636

From: Robison, Timothy W
Sent: Monday, November 19, 2007 9:32 AM
To: Robison, Timothy W; Atrakchi, Aisar H; Elespuru, Rosalie K.; Levy, Dan; Jacobson-Kram, David; Heflich, Robert; Bigger, Anita; Ouyang, Yanli; Moore, Martha; Agarwal, Rajiv; Benz, Robert Daniel; De, Mamata; Jagannath, Devaraya R; McGovern, Timothy J; Nostrandt, Amy C; Sheu, Chingju W; Sotomayor, Rene E; Yao, Jiaqin
Cc: Hausner, Elizabeth A
Subject: CDER Genetic Toxicology Subcommittee Consult

Please see the attached request for consult from the CardioRenal Division. Questions from the Reviewer are in the attached word document. These appear to be fairly complex questions. Please send responses, comments, and questions to the entire committee and reviewer. Thank you.

Tim Robison

From: Bigger, Anita
Sent: Monday, November 19, 2007 9:11 AM
To: Robison, Timothy W; Hausner, Elizabeth A
Cc: Bigger, Anita
Subject: FW: question for the genetox committee

Hi Elizabeth,

I'm forwarding your request to Tim Robison, as he is the chair of the committee now and handles requests for consults.

Anita

From: Hausner, Elizabeth A
Sent: Monday, November 19, 2007 8:53 AM
To: Bigger, Anita
Subject: question for the genetox committee

Hi Anita,
there is a genetox question in an NDA that I'm working on and I would like to get an opinion from the GC. I have a summary prepared and was wondering about whether the committee could review it and give opinions by email. Possible? << File: genotox_summary.doc >>
The summary is attached. If there is another procedure that should be used please let me know. thank you in advance,
Elizabeth

EMAIL SERIES 2

Radio labeled studies are done all the time in human volunteers. The radiation doses are negligible.

David Jacobson-Kram, Ph.D., D.A.B.T.
Office of New Drugs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
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Dan D. Levy, Ph.D.
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To: Robison, Timothy W; Atrakchi, Aisar H; Elespuru, Rosalie K.; Levy, Dan; Jacobson-Kram, David; Heflich, Robert; Bigger, Anita; Ouyang, Yanli; Moore, Martha; Agarwal, Rajiv; Benz, Robert Daniel; De, Mamata; Jagannath, Devaraya R; McGovern, Timothy J; Nostrandt, Amy C; Sheu, Chingju W; Sotomayor, Rene E; Yao, Jiaqin
Cc: Hausner, Elizabeth A
Subject: CDER Genetic Toxicology Subcommittee Consult

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Cc: Bigger, Anita
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Subject: question for the genetox committee

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The summary is attached. If there is another procedure that should be used please let me know.
thank you in advance,
Elizabeth

APPENDIX 3

Summary of Calcium Channel Reproductive Toxicity from MicroMedex

Amlodipine: When amlodipine was administered to rats from day 17 of gestation through parturition, the highest dose studied, 10 mg/kg/d (compared to a human daily dose of up to 10 mg/person/d), was lethal to some dams and caused delayed parturition and dystocia in 13 of 24 exposed animals (1). Although pup survival was impaired, adverse effects on development were not detected among surviving pups. Similar findings were reported from a high dose study in pregnant mice

Based on a small number of cases with unsuccessful in vitro fertilization, the use of verapamil and other calcium channel blockers for hypertension control has been associated with reversible male infertility (3). Calcium plays important roles in sperm activation during fertilization. The inhibitory effects of calcium channel blockers on sperm can occur at concentrations 2 to 4 orders
of magnitude below the levels found in patients on long-term therapy (3). In a rat study, a 30-day treatment with amlodipine was associated with a variety of adverse effects on male fertility including decreased plasma follicle-stimulating hormone and testosterone; and significant
reductions (23%) in sperm density as well as in the number of mature spermatids (14%) and Sertoli cells (9%) counted in seminiferous tubule cross-sections. We did not locate reports on the possible effects of the calcium channel blockers on testosterone levels and related reproductive tissues in human subjects.

1. Horimoto M, Takeuchi K, Iijima M, Tachibana M: [Reproductive and developmental toxicity studies with amlodipine in rats and rabbits]. Oyo Yakuri 42:167-76, 1991.

3. Benoff S, Cooper GW, Hurley I et al: The effect of

calcium ion channel blockers on sperm fertilization

5. Almeida SA, Teofilo JM, Anselmo Franci JA, Brentegani LG, Lamano-Carvalho TL: Antireproductive effect of the calcium channel blocker amlodipine in male rats. Exp Toxicol Pathol 2000;52:353-6.potential. Fertil Steril 62:606-17, 1994.

Diltiazem : Updated 10/1/2004
can cause abnormal embryo development in experimental animals.
Diltiazem decreases myometrial activity in the rat (2,3) and this and other calcium channel blockers, such as verapamil, have been used successfully to stop human preterm labor (4,15).
Diltiazem has been found to increase the motility of human sperm in vitro (6). Based on a small number of cases with unsuccessful in vitro fertilization, the use of verapamil and other calcium antagonists for hypertension control has also been associated with reversible male
infertility (13). Calcium plays important roles in sperm activation during fertilization. The inhibitory effects of calcium antagonists on sperm can occur at concentrations 2 to 4 orders of magnitude below the levels found in patients on long-term therapy (13). Some investigators have challenged the conclusion that calcium antagonists can impair male fertility in the absence of other factors (17).

2. Abel MH, Hollingsworth M: Comparison of nifedipine and diltiazem with salbutamol for prevention of preterm delivery in the ovariectomized, oestrogen-treated late pregnant rat. J Reprod Fertil 77:559-68, 1986.

3. Abel MH, Hollingsworth M: The potencies and selectivities of four calcium antagonists as inhibitors of uterine contractions in the rat in vivo. Br J Pharmacol 85:263-9, 1985.

4. Stix J et al: [Late cardiac sequelae in children following long-term tocolysis with fenoterol and verapamil]. Geburtshilfe Fraunheilkd 42:857-61, 1982.

6. Hong CY et al: Calcium antagonists stimulate sperm motility in ejaculated human semen. Br J Clin Pharmacol 19:45-9, 1985.

13. Benoff S, Cooper GW, Hurley I et al: The effect of calcium ion channel blockers on sperm fertilization potential. Fertil Steril 62:606-17, 1994.

15. El-Sayed YY, Holbrook RH Jr, Gibson R, Chitkara U, Druzin ML, Baba D: Diltiazem for maintenance tocolysis of preterm labor: comparison to nifedipine in a randomized trial. J Matern Fetal Med 1998;7:217-21.

17. Katsoff D, Check JH: A challenge to the concept that the use of calcium channel blockers causes reversible male

infertility. Hum Reprod 1997;12:1480-2.

Felodipine: updated 6/1/2006 Felodipine causes limb defects in experimental animals. Case reports of limb defects in humans with antenatal exposure to calcium channel blockers. Not known if there is a causal relation.

Teratology studies in rabbits have shown an increase in digital defects in the offspring of treated does (1-3). These defects consist of reduction, absence, or malformation of the distal phalanges, associated with abnormal cartilage differentiation and ossification. It is believed that the abnormalities are secondary to decreased uteroplacental blood flow rather than a direct effect of
the drug on the phalanges (2,3). According to the manufacturer (Merck Sharp & Dohme, West Point PA), teratology studies in monkeys showed abnormally positioned distal phalanges in the offspring, without reduction defects of the digits. The manufacturer also reports a delay in parturition in rats given this agent. This result is consistent with a decrease in contractility produced by calcium channel blockers in myometrium taken from pregnant human uteri at cesarean section (4).

In a fertility study reported by the manufacturer, male and female rats treated with as much as 26.9 mg/kg/d felodipine had normal reproductive function. In chronic studies, male rats given this agent showed a decrease in testosterone, an increase in luteinizing hormone (LH), and
the appearance of benign Leydig cell tumors. The latter was believed due to the elevation of LH. Based on a small number of cases with unsuccessful in vitro fertilization, the use of verapamil and other calcium antagonists for hypertension control has also been associated with
reversible male infertility (6). Calcium plays important roles in sperm activation during fertilization. The inhibitory effects of calcium antagonists on sperm can occur at concentrations two to four orders of magnitude below the levels found in patients on long-term therapy (6).

6. Benoff S, Cooper GW, Hurley I et al: The effect of calcium ion channel blockers on sperm fertilization potential. Fertil Steril 62:606-17, 1994.

Isradipine: revised 4/1/2003 Structurally related to nifedipine. Decreases uterine contractility at doses that are used for hypertension. Same comments about sperm.

Nicardipine: 2/1/2005 inhibits uterine activity in rabbit and rat and causes decrease in uteroplacental blood flow. Same comments about sperm.

Nifedipine: 2/1/2006 Teratology studies in rabbits have shown an increase in digital defects in the offspring of does treated with nifedipine, nitrendipine (#2642), felodpipine (#3392), and

hydralazine (#1522) (34,35). These defects consisted of reduction, absence, or malformation of the distal phalanges, associated with abnormal cartilage differentiation and ossification. It is believed that the abnormalities were secondary to decreased uteroplacental blood flow rather than a direct effect of the drug on the phalanges; however, experiments in rats suggest that
hyperphalangism produced in the offspring by maternal nifedipine may be due to effects on calcium channels of the limb bud mesenchymal cells (36,37).

Nimodipine: 11/1/2003 similar to others in class

Nisoldipine:

Verapamil: 9/1/2002 A group of cardiologists reported three cases of impotence that developed among 14 males receiving long-term treatment with verapamil for atrial and supraventricular arrhythmias (17). One patient discontinued his verapamil intake and returned to normal sexual functioning. When he again took verapamil, impotence recurred. These observations suggest a relationship between verapamil and impotence for some men. Based on a small number of cases arising from unsuccessful in vitro fertilization, the use of verapamil and other calcium antagonists for hypertension control has also been associated with reversible male infertility (28). Calcium plays important roles in sperm activation during fertilization. The inhibitory effects of calcium antagonists on sperm has been shown to occur at concentrations 2 to 4 orders of magnitude below the levels found in patients on long-term therapy (28).

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Elizabeth Hausner
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Elizabeth Hausner

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PHARMACOLOGIST

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 65114
Review number:
Sequence number/date/type of submission: 059 annual report , 062 IB
Correspondence date: August 30, 2006; September 20, 2006
Center Receipt Date:September 1, 2006; September 22, 2006
Reviewer Receipt Date: November 1, 2006 ; November 1, 2006
Information to sponsor: Yes (x) 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: December 6, 2006

Drug:clevidipine

Relevant INDs/NDAs/DMFs: no others

Drug class: calcium channel blocker antihypertensive
Intended clinical population:

Clinical formulation: emulsion in 20% lipid

Route of administration: IV

Proposed clinical protocol: none

Previous clinical experience: Clevidipine was developed by Astra Hassle of Molndal, Sweden and all preclinical work was done by them in the mid-1990s. Clevidipine is an emulsion of 0.5 mg/ml in 20% lipid that is dosed by titration to blood pressure lowering effect. The duration of dosing is limited to thus also limiting the duration of dosing to a

Studies reviewed within this submission: Although this is an annual report, the sponsor included several studies.

- Salmonella-Escherichia coli/Mammalian-Microsome Reverse mutation assay with a confirmatory assay with clevidipine
- Salmonella/ Mammalian-microsome reverse mutation assay in the presence of formaldehyde dehydrogenase with a confirmatory assay with clevidipine
- A continuous intravenous infusion teratology study of clevidipine in the rabbit
- A continuous intravenous infusion toxicity study of clevidipine in nonpregnant New Zealand White rabbits
- A continuous intravenous infusion pre and postnatal study of clevidipine in the rat
- A 14-day intravenous infusion toxicity study of clevidipine in the male Beagle dog with a 28-day recovery period.

Studies not reviewed:

All studies submitted were reviewed.

Salmonella-Escherichia coli/Mammalian-Microsome Reverse mutation assay with a confirmatory assay with clevidipine

Study report: —— # 7616-100
Study location: [redacted]
Genetic Tox Assay Number: 26599-0-409OECD
Study dates: initiated Sept 22, 2004
GLP: statement included
QA: yes
Test article: clevidipine, lot number 2930.D.03.5

The tester strains used in the mutagenicity assay were Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537, and E.coli tester strain WP2uvrA. The assay was conducted both ±S9 along with concurrent vehicle and positive controls using 3 plates per dose. The concentrations tested in the initial mutagenicity assay with all tester strains both ±S9 were 10.0, 33.3, 100, 333, 1000, 2000 and 5000 µg per plate. A

confirmatory, independent assay was conducted using the same doses. Additional doses of 1500, 2500, 3000 and 4000 µg per plate were tested with TA98 and TA100 in the presence of S9 to confirm observations made in the initial assay.

Positive controls are summarized in the copy of the sponsor's table below:

Positive controls			
Tester Strain	S9 mix	Positive Control	Dose (µg/plate)
TA98	+	Benzo[a]pyrene	2.5
TA98	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracence	2.5
TA100	-	Sodium azide	2.0
TA1535	+	2-aminoanthracence	2.5
TA1535	-	Sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	-	ICR-191	2.0
WP2uvrA	+	2-aminoanthracene	25.0
WP2uvrA	-	4-nitroquinoline-N-oxide	1.0

Results

In the range-finding assay:

TA100 + S9: ≥3300 µg reduced lawn and precipitate
TA100 –S9: ≥333 µg normal lawn with precipitate

WP2*uvr*A +S9: 5000 µg normal lawn with precipitate
WP2*uvr*A -S9: ≥333µg normal lawn with precipitate

In the confirmatory mutagenicity assay, no valid data were generated with tester strain WP2*uvr*A due to contamination in all plates for this strain. The test article was re-tested with WP2*uvr*A in 26599-D1. No increase in revertants was apparent in the data.

TA100 +S9: Sponsor's assessment was that the assay showed a 3.4-fold positive increase in the mean number of revertants per plate. I agree that a concentration-related increase in revertants was seen in this strain (333-2000 µg). The increase exceeded the historical control range.

TA98 +S9: Sponsor's assessment was that the assay showed a 1.9-fold increase. Reproducible increases were seen in this strain in both the initial and confirmatory trials but did not meet the 2-fold criteria for a positive response.
A concentration-related increase in revertants was seen in this strain at the same concentrations as the increase in TA100 (333- 2000 µg). The increase did not exceed the historical control range.

Reviewer's summary of mean revertants

	Mean revertants per plate with standard deviation				
	Trial B1		Trial C1		
Conc/plate	TA98 +S9	TA100 +S9	Conc/plate	TA98 +S9	TA100 +S9
Vehicle control	21±4	109±12		25±2	97±6
			333µg	20±4	97±19
333µg	17±3	114±17	1000µg	30±14	210±5
1000µg	33±4	147±13	1500µg	35±1	310±4
2000µg	53±3	251±19	2000µg	34±4	326±51
5000µg	35±9	127±9	2500µg	35±2	199±49
			3000µg	48±17	101±43
			4000µg	26±9	67±29
Historical range	8-60	60-240			

Under the conditions of the assay, an increase in revertants was seen in TA100 +S9 and in TA98 +S9. The Ames assay that is referenced in November 30, 2004 review (serial #026) by C. Resnick, Ph.D. did not produce an increase in revertants.

Salmonella/mammalian-microsome reverse mutation assay in the presence of formaldehyde dehydrogenase with a confirmatory assay with clevidipine.

Study number: [redacted] number 7616-101
Genetic toxicology assay number: 26599-0-401OECD

Study location: [redacted]
Genetic Tox Assay Number: 26599-0-409OECD
Study dates: initiated January 26, 2005
GLP: statement included
QA: yes
Test article: clevidipine, lot number 2930.D.03.5

The tester strains used in this assay were Salmonella typhimurium TA98 and TA100 with microsomal activation (S9) ± formaldehyde dehydrogenase (FDH). Vehicle controls were plated for all tester strains in the presence of S9 mix. The DMSO vehicle control was plated using a 50 µl aliquot along with a 100 µl aliquot of the appropriate tester strain and a 500 µl aliquot of the S9 mix .

The positive controls used are summarized in this copy of the sponsor's table:

Positive controls			
Tester strain	S9 mix	Positive control	Dose (µg/plate)
TA98	+	Benzo[a]pyrene	2.5
TA100	+	2-aminoanthracene	2.5

All concentrations of test article, the vehicle controls, and the positive controls were plated in triplicate. THe FDH was used at a concentration of 0.1 units FDH/ml in the initial assay. Because the expected reduction in mean revertants was not seen, the concentration was raised to 10 units FDH/ml for the confirmatory assay. Inexplicably in view of the sponsor's hypothesis, formaldehyde was not used as one of the controls.

Results

Sponsor's interpretation of preliminary assay:

TA100 +FDH: a 3.4-fold increase in mean revertants per plate
TA100-FDH:1.7fold increase
TA98-FDH:1.7 fold increase
TA98+FDH: 1.9-fold increase

Sponsor's interpretation of confirmatory assay:

TA100 +FDH: 2.0-fold increase in mean revertants
TA100-FDH: 2.0-fold increase in mean revertants
TA98-FDH: 2.1-fold increase
TA98+FDH: 1.95-fold increase

Consistent with the previous Ames assay, TA98 and TA100 showed repeatable increases in mean revertants. The increases in the TA98 strain do not show a convincing correlation with concentration. As stated in Dr Resnick's review, the sponsor has stated that clevidipine is metabolized to an inactive metabolite and formaldehyde. Genotoxic effects in the mouse lymphoma and chromosome aberration tests were originally attributed to the formation of formaldehyde. It was hypothesized that the addition of formaldehyde dehydrogenase would cause a decrease in revertants.

In the TA98 strain, there was no apparent difference in mean numbers of revertants with the addition of FDH. The revertants seen in the positive control plates decreased with FDH present.
To increase the clarity of results, I have re-organized the sponsor's table.

Reviewer's summary of mean revertants

	TA98 -FDH +S9				TA98 +FDH +S9			
Trial(replicate)	1	2	3	mean	1	2	3	mean
Vehicle control	26	24	25	25	24	29	21	25
33 µg	24	23	24	24	14	33	21	23
100 µg	15	23	34	24	24	18	26	23
333µg	27	27	24	26	9	22	19	17
1000 µg	35	43	49	42	36	42	40	39
1500 µg	45	37	36	39	53	42	45	47
2000µg	30	38	32	33	20	32	26	26
2500 µg	14	18	19	17	28	21	8	19
3000 µg	40	42	35	39	42	28	35	35
4000 µg	24	34	22	27	15	27	12	18
5000 µg	20	17	13	17	11	19	21	17
Positive control	438	386	551		107	277	60	

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In strain TA100, there was an increase in mean revertants with the addition of FDH. This increase occurred in the same concentration range as the increase seen without FDH. While this may be the normal variability of the assay, this does not seem to be consistent with the sponsor's hypothesis that the apparent genotoxicity is due to the formaldehyde metabolite.

Reviewer's summary of mean revertants

	TA100 -FDH +S9				TA100 + FDH +S9			
Trial(replicate)	1	2	3	Mean	1	2	3	Mean
Vehicle control	129	101	116	115	78	86	100	88
33 µg	89	125	126	113	68	85	98	84
100 µg	94	94	113	100	91	95	87	91
333µg	115	97	116	109	81	84	81	82
1000 µg	150	168	156	158	174	194	192	187
1500 µg	172	215	211	199	247	204	242	231
2000µg	80	107	122	103	168	392	334	298
2500 µg	77	75	142	98	414	162	180	252
3000 µg	79	67	65	70	171	107	113	130
4000 µg	44	55	61	53	77	77	77	77
5000 µg	79	53	63	65	57	54	59	57
Positive control	1421	1582	1522	1508	1094	1434	1036	1188

The results of this assay are not consistent with the sponsor's hypothesis that the use of formaldehyde dehydrogenase should decrease the number of revertants produced.

A continuous intravenous infusion toxicity study of clevidipine in nonpregnant New Zealand White rabbits

[redacted] project number: 900624
Study location: [redacted]
GLP: statement included
QA: no
Study initiated: dosing commenced March 9, 2005
Test article: clevidipine lot# KV1348

The point of this study was to evaluate the feasibility of dosing Intralipid 20% as a vehicle and clevidipine formulated in Intralipid to rabbits for the definitive teratology study. The text reports that in a previous dose-ranging study (Astra 96160) and a teratology study (Astra 97010) performed using Dutch rabbits, a "significant" number of rabbits died, were euthanized or aborted their pregnancies at all doses of clevidipine and in the Intralipid control group. The high dose infusion rate was 0.92 ml/kg/hour administered for 24 hours. The current study duplicated the doses of Intralipid and clevidipine used previously.

NZW rabbits ~5 months of age at the start of the study were used. A total of 5 rabbits were used The animals were implanted with catheters approximately 1 week after arrival. Catheters were surgically implanted via the femoral vein into the vena cava and exited subcutaneously through the nape of the neck. A jacket and tether system was used to protect the catheter.

Group	Dose (mg/kg/day)	Infusion rate (ml/kg/hour)	# of females
Intralipid 20%	0	0.92	3
clevidipine	55	0.92	2

Body weight and food consumption were measured daily throughout the study.
Observations for clinical changes were also made daily.
Animals were euthanized Day 14. Complete gross examination of the carcass was performed. No tissues were retained.

Results

There was no unscheduled mortality.

One female receiving the Intralipid received only 20% of the intended dose on Day 8 due to a kinked catheter. The doe had received ±10% of the intended dose for the other 13 days of the study so this deviation probably did not have a significant effect upon the results.

A difficulty of interpretation is that there was no untreated group and no saline infusion group for comparison purposes. There were no significant differences between the clevidipine and Intralipid groups as far as weight gain.

Summary selected group mean body weights (kg)

	Day			
	1	7	10	14
Intralipid group	3.20±0.252	3.27±0.219	3.33±0.285	3.37±0.318
Clevidipine	3.15±0.250	3.25±0.250	3.25±0.350	3.30±0.400

The animals in both groups were offered 180 g of food per day. In the records provided, neither group of animals consumed the full amount offered. This may have been due to calories provided by the Intralipid or general malaise due to the catheters.

Only 2 gross observations were reported. One observation was a raised area in the lung of a clevidipine rabbit. The other observation was a dark focus on the thymus of an Intralipid rabbit. The pathologist's report stated that no treatment related lesions were apparent.

Summary: in this study, non-pregnant NZW rabbits received either intravenous Intralipid or intravenous clevidipine formulated in Intralipid for 14 days. There was no unscheduled mortality. No gross lesions were reported. We do not have an untreated control group or a saline infusion group to compare weight gain and food consumption.

A continuous intravenous infusion teratology study of clevidipine in the rabbit.

Project number: 900591
Study location: [redacted]
GLP: statement included
QA: yes
Study initiated: Dosing began April 11, 2005
Test article: clevidipine lot# KV1348
Test article was given intravenously from GD7 to GD19 of gestation

The report references a dose range finding study (Astra 96160) and a main teratology study (Astra 97010) conducted at another laboratory with Dutch rabbits. It is reported that in both those studies a significant number of rabbits died, were euthanized ahead of schedule or aborted the pregnancies at all doses of clevidipine and in the intralipid control. An exploratory study using non-pregnant NZW rabbits was conducted and is reviewed in this submission. The study with non-pregnant NZW suggested that this strain was less sensitive to the effects of the intralipid vehicle.

Catheters were inserted approximately 1 week before insemination. Catheters entered via the femoral artery and were placed in the vena cava. The end of the catheter was exteriorized over the nape of the neck. A jacketed tether system was used to protect the catheters.

	Dose mg/kg/day	Conc Mg/ml	Infusion rate Ml/kg/h	# of females
Saline control	0	0	0.92	25
Intralipid control	0	0	0.92	25
Clevidipine	13	0.59	0.92	25
Clevidipine	35	1.59	0.92	25
clevidipine	55	2.49	0.92	25

Untreated proven bucks were used to provide samples for the artificial insemination.

Animals were examined twice a day for signs.
Body weight was determined gestation days 0, 4,7,10, 13,16, 20, 23, 26 and 29. Food consumption was measured daily during gestation.
GD29: gross pathological examination was performed for all animals, tissues were retained and the reproductive tract of each female was dissected out, the ovaries removed and the corpora lutea counted. The gravid uterus was weighed, uterine contents examined, live and dead feti, early and late resorptions. Ammonium sulfide was used to determine implantation sites in non-pregnant females.

Fetuses were weighed, examined and euthanized. Heads of approximately ¼ were fixed in Bouin's. Eviscerated feti were preserved, stained with alizarin red s and prepared for skeletal examination.

Results

Unscheduled mortality: found dead or euthanized for poor condition between GD12-24
1 saline control (severe skin lesion)
1 intralipid control: found dead
2 from 13 mg/kg/day: euthanized after 5 days poor food consumption
1 from 35 mg/kg/day: found dead
1 from 55 mg/kg/day : euthanized, not pregnant

Macroscopic findings from the stomach and/or colon of 1 LD f and 1 HD f were described as possibly linked to deteriorating condition.

Clinical signs in survivors:

Reduced appetite, decreased weight, skin pallor, absent /decreased/soft/liquid feces
Staining of anogenital areas
Abortions

The abortion rate in the HD group was 30.4%, above the historical control range of 0-18%.

Observation	Treatment group				
	Saline	intralipid	LD	MD	HD
Abortions/total does (%of total)	0/22	1/21	1/23	3/21 (14.3%)	7/23 (30.4%)
#total absorptions	0	0	0	1	2
#dieing/euthanized	1	1	2	1	1
Feces absent	0	3	6	5	15
Liquid feces	0	1	4	4	6

Mean body weight gains

There was a slight decrease in body weight gain due to the lipid vehicle. There was an apparent drug-related decrease in body weight gain at the MD and a slight weight loss at the HD at the end of the dosing period. After the end of the dosing period, all groups gained weight, although the overall gains were lowest in the MD and HD groups.

Summary of mean maternal body weight gains (kg)

Period of measurement	Treatment group				
	saline	intralipid	LD	MD	HD
GD7	3.38	3.50	3.47	3.51	3.51
GD20	3.66	3.66	3.70	3.56	3.45
ΔGD7-20	0.28	0.16	0.23	0.05	-0.06
GD29	3.88	3.91	3.96	3.87	3.75
ΔGD7-29	0.50	0.40	0.49	0.36	0.24

Food consumption was also decreased in some groups. Decreased consumption began
GD7 in the HD group
GD8 in the MD group
GD11 in the intralipid vehicle group
GD12 for the LD group

Sponsor's summary of poor food consumption

group		% pregnant females/group with food consumption <70g for 1 or more days	Mean number of days/pregnant females with food consumption≤70 g
Saline control		23%	2.2
20% intralipid control		81%	4.8
Clevidipine Mg/kg/day	13	78%	5.3
	35	100%	6.1
	55	100%	9.4

Pregnancy/uterine parameters
There were no apparent effects on the total implantation sites, ratio of male:female feti, early or late resorptions.

There seems to be a vehicle effect with regard to pre-implantation loss. When animals with total resorptions are included, there is a dose-related increase in post-implantation loss. The weight of the gravid uterus was decreased slightly in the intralipid group and showed further decrease in the drug-treated groups.

Summary of pre and post-implantation loss and gravid uterine weight

group	Pre-implantation loss %	Post-implantation loss %	Gravid uterus weight (g)
Saline control	7.9±7.16	4.4±7.58	553.4±69.61
Intralipid control	17.8±16.62	8.4±20.19	457.2±144.55
LD	21.6±25.44	5.5±10.83	433.6±151.03
MD A	10.6±9.52	15.4±24.56	443.8±104.84
MD B	10.7±9.82	10.2±11.71	443.8±104.84
HD A	20.0±20.68	20.3±31.97	399.6±129.40
HD B	19.3±22.01	9.6±11.78	399.6±129.40

A= including animals with total resorptions
B= excluding animals with total resorption

Mean fetal weight was decreased in the intralipid control group and further decreased in the drug-treated groups as summarized below. There was no difference in effect on the two sexes of the pups, so only the group means are presented.

Summary of mean fetal weight

	saline	intralipid	LD	MD	HD
Mean fetal weight	44.3 ±4.14	40.1 ±7.53	40.9 ±7.45	39.9 ±6.52	38.9 ±9.43

Fetal anomalies, variants, malformations

There was a slight increase in litters affected by incomplete ossification of the hyoid bone:

Saline: 5litters, intralipid: 8, LD: 8,MD: 6, HD:8 litters.

Summary: Overall, there was no clear trend of increased anomalies with treatment. The sponsor concluded a developmental NOAEL of 35 mg/kg/day. No maternal NOAEL was identified. The effects upon parturition (dystocia, delayed parturition) are consistent with effects identified with other calcium channel blockers due to the role of calcium in muscle contraction.

A continuous intravenous infusion pre and postnatal study of clevidipine in the rat

Report number: 900510
Study location: [redacted]
Study initiated: dosing began January 24, 2005
GLP compliance: statement included
QA: yes
Test article: KV1348 in 20% Intralipid emulsion

A previous study in rats administered a HD of 66 mg/kg by continuous infusion, resulting in a blocked cannula (Study #96008). Continuous infusion teratology study 97003 was referenced as successfully conducted with a HD of 55 mg/kg. Decreased food consumption and increased preimplantation losses were seen primarily at the HD. A dose related increase in renal cavitation was also reported above the effects of the vehicle. Continuous infusion fertility study 97049 was also referenced as successfully conducted with a HD of 55 mg/kg. The doses chosen for this study were based upon the previous experiences. Clevidipine at levels of 13, 35 and 55 mg/kg/day were used.

Samples of the dosing formulations were collected for analysis day 1 and during the last week.

The sponsor tested the effect of clevidipine upon gestation, parturition and lactation by administering the drug to the dams from Day 6 of gestation to the morning of post-partum day 4. The doses used are summarized in the table below.

Summary of study design

group	Dose (mg/kg/day)	Concentration(mg/ml)	# of animals(dams)
Saline control	0	0	24
Intralipid control	0	0	24
clevidipine	13	0.6	24
clevidipine	35	1.6	24
clevidipine	55	2.5	24

Surgery was performed Day 0 of gestation. Catheters were inserted through the femoral vein into the vena cava. The end of the catheter was exteriorized at the nape of the neck. A jacket and tether system was used to protect the catheters.

Animals were observed twice daily for signs. Body weights were determined days 0, 3,6,9,12,15,18 and 20 of gestation and days 0,4,7,14,17 and 21 post partum.
The day of completion of littering was called PP0. Maternal behavior (F0)was observed immediately post partum.

F1 pups:

examined PP0 for malformations, sex, mortality, and weighed. Pups found dead were either examined immediately or placed in Bouin's fixative for subsequent evaluation.

Pups were weighed days 4,7,14 and 21.

Day 4, litters were culled to 8 pups where necessary to give a litter of 4 males and 4 females where possible. Culled pups were not examined.

Day 21, pups were separated from the dams. Those not selected to form the adult generation were given a gross pathological examination.

Summary of F1 developmental landmark assessment

parameter	Day of assessment
Pinna unfolding	Days 1-4
Tooth eruption	From day 7
Eye opening	From Day 12
Righting reflex	Days2-4
Negative geotaxis	From day 8
Auricular startle	From day 12
Vaginal opening	Day 26
Preputial separation	Day 35
Visual function	Day 21
Locomotor activity	Days 35 ±1and 60±2
Auditory startle habituation	Day 55±2
E water maze	Between days 60 and 70

Selection of F1 adult generation

Around the time of weaning, 2 male and 2 female rats were selected from each litter where possible to provide the F1 adult generation. Each litter provided 1 pup/sex to each of 2 subsets: subset I, subset II. Subset I was originally to be used for behavioral assessment. Subset II was to be used for the reproductive phase. Because of the shortage of animals, those in subset II were also included in the behavioral assessments.

F1 adults body weight determination

Individual body weights were measured weekly in the premating period. Mated females were weighed days 0,6,9,12,15,18 and 20 of gestation and on days 0 and 4 post-partum.

F1 mating procedures

At ~85 days of age, 1 female was placed with 1 male in the same dosage group for a maximum of 14 days. Mating was determined by examination of vaginal lavage for spermatozoa. Day of identification of spermatozoa was called day 0 of gestation. Dams were observed for parturition. The pups (F2) were examined for malformations, sex and mortality.

Some tissues were collected. I am not clear from the report from which generations these tissues were retained:
Abnormalities
Epididymides
Mammary glands
Ovaries
Prostate
Seminal vesicles
Testes
Uterus
Vagina

Results

F0 females
55 mg/kg: All females either found dead or were euthanized
6 females were found dead.
18 females were euthanized primarily due to dystocia, prolonged parturition
And/or poor or deteriorating condition
There were no pups from this group for evaluation of the F1 generation

35 mg/kg: 1 female found dead
18 euthanized primarily due to dystocia, prolonged parturition
And/or poor or deteriorating condition
Only 5 dams had viable pups on PP4

13 mg/kg: 3 females were found dead
6 females euthanized primarily due to dystocia, prolonged parturition,
poor and deteriorating condition and/or failure to litter.
Only 13 dams had viable litters

Intralipid: 2 females found dead in mid-gestation
4 females euthanized due to prolonged parturition, failing to litter or
Cannibalism.

Saline control: 2 euthanized day 24 post-breeding were not pregnant

Clinical signs for Intralipid and drug-treated groups included dose-dependent increases in abnormal gait, decreased activity, weight loss, tachypnea, shallow breathing, ptosis, dehydration, hunching, decreased muscle tone, fur erect and ungroomed, staining of muzzle, periorbital and urogenital fur.

Gross lesions for the F0 females were summarized by the sponsor as shown below:

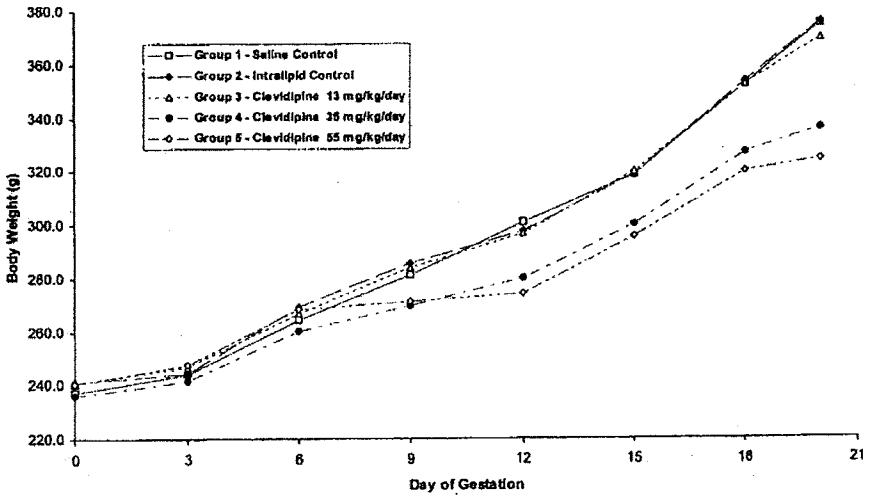
	Saline control	Intralipid control	Clevidipine (mg/kg/day)		
			13	35	55
#animals examined	24	24	24	24	24
Infusion site: swelling, pale fluid +/or material,+/or masses	0	4	7	9	9
Lungs: mottled dark areas+/or foci, pale or dark discoloration +/or depression	4	9	12	17	18
Liver: pale or dark foci, discoloration, raised or depressed area +/or enlargement	0	4	4	7	13
Adrenal glands: enlargement with pale or dark foci, discoloration	0	4	8	13	15
Enlarged spleens	0	5	5	5	9

Reviewer's summary of Necropsy findings for F0 generation based on summary tables

Organ/finding	Saline control	Intralipid control	Clevidipine mg/kg/day		
			13	35	55
Adrenal enlargement	-	3	8	12	15
Liver: area dark, pale depressed, or raised	-	1	2	3	10
Liver: dark or pale discoloration, dark or pale foci	-	6	3	10	6
Lung: dark area	3	6	7	8	13
Lung: dark focus	-	2	4	6	6
Lung: pale discoloration	-	2	-	3	7
Spleen: enlarged	-	5	5	5	9

Body weight gain was affected from Day 6 of gestation through the end of the study for the F0 generation. See sponsor's graph below.

Figure 1 **Group Mean Body Weights (g) of Females During Gestation - F0 Generation**



Summary of group mean body weights(g) of F0 females during gestation

group	ΔGD6-GD20 (gestation)
Saline control	110.5
Intralipid control	105.9
Clevidipine 13 mg/kg/day	102.8
Clevidipine 35 mg/kg/day	75.4 $^{a\,B}$
Clevidipine 55 mg/kg/day	55.9$^{c\,C}$

significantly different from vehicle control a= $p<0.05$, b=$p<0.01$, c=$p< 0.001$
significantly different from saline control B= $p< 0.01$, C= $p<0.001$

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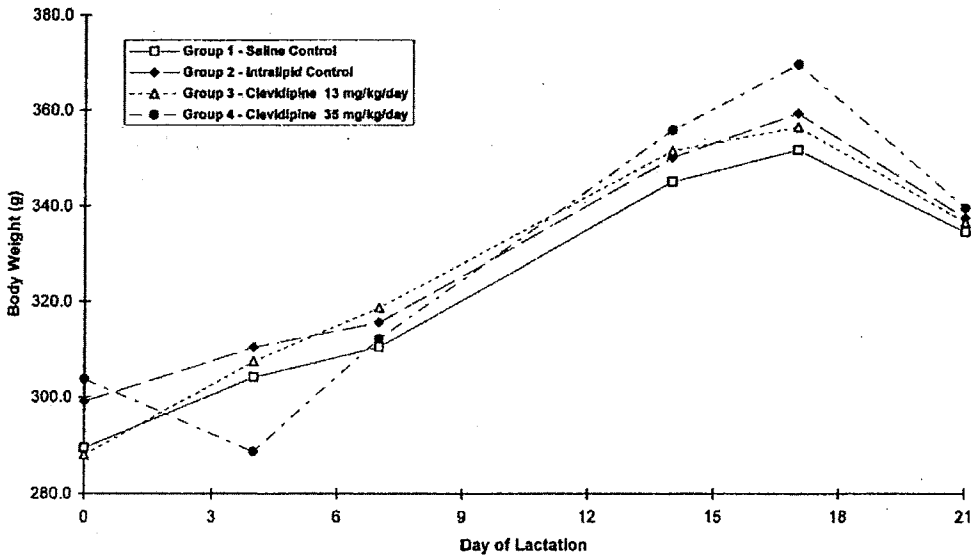
The drug-treated dams gained more than the saline control during lactation.

gure 2 **Group Mean Body Weights of Females During Lactation - F0 Generation**



Summary of group mean body weights(g) of F0 females during gestation and lactation

group	ΔPP0-PP21(lactation)
Saline control	45.2
Intralipid control	38.4
Clevidipine 13 mg/kg/day	48.7
Clevidipine 35 mg/kg/day	35.8
Clevidipine 55 mg/kg/day	No survivors

significantly different from vehicle control a= $p<0.05$, b=$p<0.01$, c=$p< 0.001$
significantly different from saline control B= $p< 0.01$, C= $p<0.001$

The sponsor's summary of maternal (F0) performance is reproduced below. No NOAEL was identified.

Sponsor's summary of maternal performance

dose	Gestation index(%)	Gestation length (days)	Live litter size	Avg # dead pups /litter	Live birth index (%)
Saline control	100.0	21.3	13.2	0.05	93.6
Intralipid	79.2	21.4	12.8	0.68	89.2
Clevidipine 13 mg/kg/day	69.6#	21.7#	10.5#,@	0.94	75.1#
Clevidipine 35 mg/kg/day	63.6#	22.1#,@	5.3#,@	0.93	39.6#,@
Clevidipine 55 mg/kg/day	9.1#,@	22.6#,@	2.5#,@	1.50	22.2#,@
HCD	95.0-100.0	21.6-22.0	13.0-15.6	0.0-0.47	89.0-94.9

HCD= historical control data
#= significantly different from saline control group
@=significantly different from the Intralipid control group

The F1 viability index showed a dose-related decrease. However, day 7 survival index was unaffected across groups.

	saline	intralipid	Clevidipine 13 mg/kg	Clevidipine 35 mg/kg
Viability index day 4	97.7±3.50 N=22	90.0±25.09 N=19	86.5±30.60 N=14	78.3±40.21 N=6

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There was no difference between F1 pups in group mean values for pinna unfolding, development of righting reflex, negative geotaxis, auricular startle, mean preputial separation, group mean activity. There was a slight decrease in time to tooth eruption, and eye opening. There was a slight increase in time to vaginal opening.

Summary of F1 developmental differences

	saline	intralipid	Clevidipine 13 mg/kg	Clevidipine 35 mg/kg
Males: day of tooth eruption	11.1±0.80	11.0±1.31	10.9±1.13	10.6±1.08
Females:day of tooth eruption	11.3±0.78	11.3±0.64	10.8±1.02	10.0±0.70Yy
Males: day of eye opening	14.2±0.67	14.2±0.47	13.6±0.63Xy	13.6±0.96
Females: day of eye opening	14.1±0.62	14.1±0.50	13.6±0.60x	13.5±0.62X
Day of Vaginal opening	33.5±1.12	33.8±1.50	33.9±1.58	34.5±2.37

Significantly different from saline at Y= $p< 0.01$, X= $p<0.05$
Significantly different from Intralipid y = $p<0.01$, x=$p<0.05$

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ON ORIGINAL