control group levels used for evaluation purposes were within historical control ranges, and 4) there was no evidence of a dose-dependent alteration in the number of revertants per plate.

Study outcome: The revertant frequencies for all doses of tetracaine base, both with and without S9, were comparable to or less than the vehicle controls. The confirmatory assay produced similar results. In the confirmatory study, the mean vehicle control value for tester strain TA1537 with S9 was above acceptable limits and therefore not-scored. The effects of tetracaine base were re-evaluated under these conditions with no evidence of mutagenicity. The results of the definitive study are reproduced below from sponsor's table 4.

Table 4 : Mutagenicity Assay Results – Summary

Test Article ID: Tetracaine Base

Experiment ID: 23841-B1

Date Plated: 11-Jun-02 Vehicle: DMSO

Date Counted: 17-Jun-02 Plating Aliquot: 50 µL

		Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
	Dose/Plate	TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		20	2	92	6	11	2	10	3	22	7	N
Test Article	33.3 µg	22	6	99	11	13	3	10	2	22	5	N
	100 µg	23	6	78	8	13	6	12	2	17	4	N
	333 µg	19	2	74	5	13	1	10	2	20	6	N
	1000 µg	20	5	84	5	12	4	8	1	12	6	N
	3330 µg	15	3	42	4	8	3	5	2	4	2	N/R[d]
	5000 µg	5	2	8	2	7	5	6	4	4	1	N/R[d]
Positive Control[b]		404	56	381	68	114	10	109	16	638	44	N
Microsomes: None												
Vehicle Control		11	2	77	6	38	8	7	2	16	7	N
Test Article	33:3 µg	10	1	90	18	47	6	7	3	17	4	N
	100 µg	9	2	108	5	37	8	12	2	12	2	N/R[e]
	333 µg	14	4	98	6	28	1	6	3	13	4	N/R[e]
	1000 µg	9	1	73	21	36	14	5	1	9	3	N/R[f]
	3330 µg	9	7	0	0	0	0	0	0	5	2	R
	5000 µg	0	1	0	0	0	0	0	0	1	1	R
Positive Control[c]		174	31	906	35	769	64	753	22	98	21	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate	WP2*uvr*A	4-nitroquinolone-N-oxide	1.0 µg/plate

[d] The first entry is the lawn evaluation for tester strains TA1535 and WP2*uvr*A.
The second entry is the lawn evaluation for tester strains TA98, TA100, and TA1537.
[e] The first entry is the lawn evaluation for tester strains TA98, TA100, and WP2*uvr*A.
The second entry is the lawn evaluation for tester strains TA1535 and TA1537.
[f] The first entry is the lawn evaluation for tester strain WP2*uvr*A.
The second entry is the lawn evaluation for tester strains TA98, TA100, TA1535, and TA1537.

Study title: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Lidocaine Base

Key findings: Under the assay conditions, lidocaine base did not produce alterations in either chromosome structure or number in CHO cells.

Study no.:	23840-0-437OECD
Volume #, and page #:	Volume 12, Page 63
Conducting laboratory and location:	[redacted]
Date of study initiation:	May 10, 2002
GLP compliance:	Yes
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	Lidocaine base, Lot # 811D0013

Methods

The effect of lidocaine base to induce chromosome aberrations in cultured Chinese hamster ovary cells was examined in vitro both with and without metabolic activation. The initial assay examined a treatment period of 3 hours with and without metabolic activation. The confirmatory assay examined the effect of lidocaine for 20 hours without metabolic activation and 3 hours with metabolic activation. Prior to harvest of the cultures, visual observations of cytotoxicity were made, including an assessment of the percent confluence of the cell monolayer and determination of the mitotic index. Following harvest of cells, only cells with good morphology with the number of centromeres equal to the modal number 21 ± 2 (range 19-23) were analyzed. One hundred cells, if possible, from each replicate culture from 4 concentrations of test article, the vehicle, negative and one dose from the positive control cultures were analyzed for chromosomal aberrations. At least 25 cells were analyzed from cultures that had greater than 25% of cells with one or more aberrations. The mitotic index was evaluated from the vehicle control and a range of concentrations by analyzing the number of mitotic cells in 1000 cells and the ratio expressed as a percentage of mitotic cells. All slides were coded prior to analysis.

The assay was considered to be acceptable by the laboratory if: 1) then negative control contains less than ~5% cells with aberrations, 2) the positive control must be significantly higher ($p \leq 0.01$) than the vehicle control, 3) there is an acceptable high concentration tested ($\geq$ 50% reduction in mitotic index) or concentration limit reached (10 mM or 5 mg/ml, whichever is lower), or the concentration exceeds the solubility and 4) there are at least 3 concentrations evaluated.

A test article was considered positive for inducing chromosome aberrations if a significant increase ($p \leq 0.01$) in the number of cells with chromosome aberrations is observed at one or more concentrations. A dose response should be observed if a significant increase was noted at one or more concentrations. The linear trend test evaluated the dose responsiveness.

Strains/species/cell line: Chinese Hamster Ovary (CHO) cells (CHO-WBL)

Doses used in definitive study: The confirmatory assay tested concentrations of 63.5, 127, 253, 505, 758, 1010, 1260, 1510 and 2010 μg/ml without metabolic activation and 253, 505, 1010, 1260, 1510, 1760, and 2010 μg/ml with metabolic activation.

Basis of dose selection: Dose selection was based on initial chromosome aberrations assay both with and without metabolic activation. Solubility of lidocaine in McCoy's 5a culture medium limited the concentrations to be tested. Lidocaine was dissolved in DMSO at a concentration of 10.0 μl/ml. At a dosed concentration of 3930 μg/ml, a precipitate formed. At 1970 μg/ml a precipitate formed but went back into solution with repeated gentle agitation for about 5 minutes and the pH was 7.5 (same as culture medium). At a concentration of 985 μg/ml, a precipitate formed that went back into solution was formed (pH 7.5). For the initial chromosome aberrations assay, concentrations of 20.4, 29.1, 41.5, 59.3, 84.7, 121, 173, 246, 351, 500, 714, 1020, 1460, 2090 and 2990 μg/ml. Reductions of 0%, 0%, 19% and 42% were observed in the mitotic indices of the cultures treated with 500, 714, 1020 and 1460 μg/ml. Chromosome aberrations were analyzed from cultures without metabolic activation treated with 500, 714, 1020 and 1460 μg/ml. At 1460 μg/ml, there was a 42% reduction in mitotic index, indicating inadequate toxicity for a valid assay. In an assay with metabolic activation, precipitation was observed with culture concentrations of 2090 μg/ml and above. Reductions in mitotic indices of 0%, 0%, 0%, 25%, 51% and 57% were observed in cultures treated with 246, 351, 500, 714, 1020 and 1460 μg/ml. Chromosome aberrations were analyzed from cultures treated with 500, 714, 1020 and 1460 μg/ml lidocaine.

Negative controls: In non-activation assay, the negative controls were cultures containing only cells and culture medium. Vehicle controls were cultures containing the vehicle for lidocaine, DMSO at 10.0 μL/ml (the highest concentration used in test cultures).

Positive controls: The positive control conditions are presented in the table below:

Assay Conditions	Treatment Duration	Positive Control	Concentration (μg/ml)
+ S9	3 hr	Cyclophosphamide (CP)	5 and 10
- S9	3 hr 20 hr	Mitomycin C (MMC)	0.75 and 1.5 0.2 and 0.4

Incubation and sampling times: CHO cells were grown in McCoy's 5a culture medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin G (100 units/ml) and streptomycin (100 μg/ml) in a humidified incubator at 37°C in an atmosphere of 5% CO_2 in air. For the assay without S9, cultures were incubated in the presence of test article for 3 hours and 20 hours. For the assay with S9, cultures were incubated for 3 hours with test article, then washed with buffered saline, re-fed with new medium, and incubated for the remainder of the culture period. For the last 2 ± 0.5 hours

of incubation, Colcemid® (0.1 μg/ml). Prior to harvest, cells were examined visually for evidence of cytotoxicity, percent confluence of the cell monolayer, presence of mitotic (large rounded cells) or dead cells floating in the medium. Cultures were then trypsonized for collection, swollen with 75 mM KCl, fixed in methanol:glacial acetic acid (3:1, v/v) fixative, stained with Giemsa solution for analysis of mitotic index and chromosomal aberrations.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears valid for the following reasons: 1) The positive controls cyclophosphamide and mitomycin C produced clear increases in chromosome aberrations; 2) the assay was conducted according to standard protocols, 3) the concentrations tested were adequate to produce a valid assay, and 4) the negative controls produced an acceptable level of chromosomal aberrations.

Study outcome: The effects of a 3 hour treatment of CHO cells lidocaine in the presence of metabolic activation are presented in the sponsor's Table 4 depicted below. As noted in Table 4, 6 and 8 on the following pages, there were no significant increases in chromosome abnormalities following a 3 or 20 hour exposure to lidocaine in the absence of metabolic activation or 3 hour exposure to lidocaine in the presence of metabolic activation, respectively.

Table 4:
Chromosome Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation – 3.0 Hour Treatment, 19.9 Hour Harvest

Assay No.: 23840 Trial No.: B2 Date: 06/12/02 Lab No.: CY7042 Test Article: Lidocaine Base

			CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS							JUDGEMENT (+/-) [d]
								Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	
CONTROLS															
NEGATIVE: McCoy's 5a		A	100		0	0							0	0	
		B	100		0	1							0	0	
		TOTAL	200										0	0	
		AVERAGE	**%**	-	**0.0**	**0.5**							**0.0**	**0.0**	
VEHICLE: DMSO	10.0µL/mL	A	100		0	0							0	0	
		B	100		0	2							0	0	
		TOTAL	200										0	0	
		AVERAGE	**%**	**0**	**0.0**	**1.0**							**0.0**	**0.0**	
POSITIVE: CP	5.00µg/mL	A	50		1	3		5	7	11	1		17	21	
		B	75		3	4		5	7	9			15	20	
		TOTAL	125					10	14	20	1		32	41	
		AVERAGE	**%**	-	**2.0**	**3.5**	-	**8.0**	**11.2**	**16.0**	**0.8**		**25.6**	**32.8**	+
TEST ARTICLE	500µg/mL	A	100		0	2		2					0	2	
		B	100		0	1		1					0	1	
		TOTAL	200					3					0	3	
		AVERAGE	**%**	**0**	**0.0**	**1.5**	-	**1.5**					**0.0**	**1.5**	-
	714µg/mL	A	100		0	0		1					0	1	
		B	100		0	2							0	0	
		TOTAL	200					1					0	1	
		AVERAGE	**%**	**25**	**0.0**	**1.0**	-	**0.5**					**0.0**	**0.5**	-
	1020µg/mL	A	100		2	2		4					0	4	
		B	100		3	2		5					0	5	
		TOTAL	200					9					0	9	
		AVERAGE	**%**	**51**	**2.5**	**2.0**	-	**4.5**					**0.0**	**4.5**	-
	1460µg/mL	A	86		3	4		1					0	1	
		B	114		1	3							0	0	
		TOTAL	200					1					0	1	
		AVERAGE	**%**	**57**	**2.0**	**3.5**	-	**0.5**					**0.0**	**0.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

Table 6:
Chromosome Aberrations in Chinese Hamster Ovary Cells - Without Metabolic Activation - 17.3 Hour Treatment, 19.8 Hour Harvest

Assay No.: 23840 Trial No.: C1 Date: 07/02/02 Lab No.: CY8032 Test Article: Lidocaine Base

			CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS							JUDGEMENT (+/-) [d]
								Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	
CONTROLS															
NEGATIVE: McCoy's 5a		A	100		0	1		2					0	2	
		B	100		0	1							0	0	
		TOTAL	200					2					0	2	
		AVERAGE	%	-	0.0	1.0		1.0					0.0	1.0	
VEHICLE: DMSO	10.0µL/mL	A	100		0	0		1	1				1	2	
		B	100		0	0		2	1				1	3	
		TOTAL	200					3	2				2	5	
		AVERAGE	%	0	0.0	0.0		1.5	1.0				1.0	2.5	
POSITIVE: MMC	0.200µg/mL	A	50		0	0		3	12	10	2	6	26	28	
		B	50		0	1		2	17	11		2	24	25	
		TOTAL	100					5	29	21	2	8	50	53	
		AVERAGE	%	-	0.0	0.5	-	5.0	29.0	21.0	2.0	8.0	50.0	53.0	+
TEST ARTICLE	127µg/mL	A	100		0	0					1		1	1	
		B	100		0	0							0	0	
		TOTAL	200								1		1	1	
		AVERAGE	%	0	0.0	0.0	-				0.5		0.5	0.5	-
	253µg/mL	A	100		0	0					1		1	1	
		B	100		0	0				1			1	1	
		TOTAL	200							1	1		2	2	
		AVERAGE	%	0	0.0	0.0	-			0.5	0.5		1.0	1.0	-
	505µg/mL	A	100		0	0		1					0	1	
		B	100		0	0							0	0	
		TOTAL	200					1					0	1	
		AVERAGE	%	10	0.0	0.0	-	0.5					0.0	0.5	-
	758µg/mL	A	100		1	0		2	1				1	3	
		B	100		0	2		1					0	1	
		TOTAL	200					3	1				1	4	
		AVERAGE	%	9	0.5	1.0	-	1.5	0.5				0.5	2.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = Dimethylsulfoxide MMC = Mitomycin C

Table 8:
Chromosome Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation – 3.0 Hour Treatment, 19.8 Hour Harvest

Assay No.: 23840 Trial No.: C1 Date: 07/02/02 Lab No.: CY8032 Test Article: Lidocaine Base

			CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS							JUDGEMENT (+/-) [d]
								Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	
CONTROLS															
NEGATIVE: McCoy's 5a		A	100		1	0		2	1				1	3	
		B	100		0	0							0	0	
		TOTAL	200					2	1				1	3	
		AVERAGE	%	-	0.5	0.0		1.0	0.5				0.5	1.5	
VEHICLE: DMSO	10.0µL/mL	A	100		0	0				1			1	1	
		B	100		0	0		3					0	3	
		TOTAL	200					3		1			1	4	
		AVERAGE	%	0	0.0	0.0		1.5		0.5			0.5	2.0	
POSITIVE: CP	5.00µg/mL	A	50		1	0		2	17	4	2		20	21	
		B	50		0	1		4	14	8		1	22	22	
		TOTAL	100					6	31	12	2	1	42	43	
		AVERAGE	%	-	0.5	0.5	-	6.0	31.0	12.0	2.0	1.0	42.0	43.0	+
TEST ARTICLE	505µg/mL	A	100		0	0			1				1	1	
		B	100		0	0		3	1				1	4	
		TOTAL	200					3	2				2	5	
		AVERAGE	%	0	0.0	0.0	-	1.5	1.0				1.0	2.5	-
	1010µg/mL	A	100		0	0		1	1				1	2	
		B	100		1	0		1					0	1	
		TOTAL	200					2	1				1	3	
		AVERAGE	%	0	0.5	0.0	-	1.0	0.5				0.5	1.5	-
	1260µg/mL	A	100		1	0		2	1				1	2	
		B	100		2	0		3	2	1			3	6	
		TOTAL	200					5	3	1			4	8	
		AVERAGE	%	0	1.5	0.0	-	2.5	1.5	0.5			2.0	4.0	-
	1510µg/mL	A	100		2	0		3	3	3			5	7	
		B	100		1	0		3		1			1	4	
		TOTAL	200					6	3	4			6	11	
		AVERAGE	%	19	1.5	0.0	-	3.0	1.5	2.0			3.0	5.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

Study title: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Tetracaine Base

Key findings: The potential clastogenicity of tetracaine base was examined in an *in vitro* chromosome aberrations assay in CHO cells with the following findings.

1. In the absence of metabolic activation, tetracaine did not produce in increase in chromosomal aberrations.
2. In the presence of metabolic activation, tetracaine base produced a significant increase in chromosomal aberrations at a concentration of 300 μg/ml, which was associated with ~85% reduction in cell monolayer confluence. At concentrations of 250 and 300 μg/ml, tetracaine produced a significant increase in endoreduplication compared to controls.
3. The equivocal findings potentially related to excessive cytotoxicity at the 300 μg/ml concentration should be further explored.

Study no.:	23841-0-437OECD
Volume #, and page #:	Volume 12, Page 97
Conducting laboratory and location:	[redacted]
Date of study initiation:	May 10, 2002
GLP compliance:	Yes
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	Tetracaine base, Lot No. 721725

Methods

The effect of tetracaine base to induce chromosome aberrations in cultured Chinese hamster ovary cells was examined *in vitro*. The initial assay employed a treatment period of 3 hours with and without metabolic activation. The confirmatory assay examined the effect of tetracaine for 20 hours without metabolic activation and 3 hours with metabolic activation. Prior to harvest of the cultures, visual observations of cytotoxicity were made, including an assessment of the percent confluence of the cell monolayer and determination of the mitotic index. Following harvest of cells, only cells with good morphology with the number of centromeres equal to the modal number 21 ± 2 (range 19-23) were analyzed. One hundred cells, if possible, from each replicate culture from 4 concentrations of test article, the vehicle, negative and one dose from the positive control cultures were analyzed for chromosome aberrations. At least 25 cells were analyzed from cultures that had greater than 25% of cells with one or more aberrations. The mitotic index was evaluated from the vehicle control and a range of concentrations by analyzing the number of mitotic cells in 1000 cells and the ratio expressed as a percentage of mitotic cells. All slides were coded prior to analysis.

The assay was considered to be acceptable by the laboratory if: 1) then negative control contains less than ~5% cells with aberrations, 2) the positive control must be significantly higher ($p \leq 0.01$) than the vehicle control, 3) there is an acceptable high concentration tested (≥ 50% reduction in mitotic index) or concentration limit reached (10 mM or 5

mg/ml, whichever is lower), or the concentration exceeds the solubility and 4) there are at least 3 concentrations evaluated.

A test article was considered positive for inducing chromosome aberrations if a significant increase ($p \leq 0.01$) in the number of cells with chromosomal aberrations is observed at one or more concentrations. A dose response should be observed if a significant increase was noted at one or more concentrations. The linear trend test evaluated the dose responsiveness.

Strains/species/cell line: Chinese Hamster Ovary (CHO) cells (CHO-WBL).

Doses used in definitive study: The confirmatory assay tested concentrations of 9.4, 18.8, 37.5, 75.0, 150, 200, 250, 300 and 350 μg/ml tetracaine in the absence of metabolic activation. Dosing for studies incorporating metabolic activation were 37.5, 75.0, 150, 200, 250, 300 and 350 μg/ml tetracaine with metabolic activation.

Basis of dose selection: Dose selection was based on initial chromosome aberrations assay both with and without metabolic activation. Tetracaine base is insoluble in water at ≥ 50 mg/ml. The solubility of tetracaine in McCoy's 5a culture medium limited the concentrations to be tested. Tetracaine was dissolved in DMSO at a concentration of 10.0 μl/ml. At a dosed concentration of 3700 μg/ml, a precipitate formed. At 1850 μg/ml a fine precipitate formed that settled down slowly in the dilution tube and was pH was 7.5 (same as culture medium). At a concentration of 925 μg/ml, a precipitate formed that went back into solution was formed (pH 7.5). For the initial chromosomal aberrations assay, concentrations of 13.7, 19.5, 27.9, 39.8, 56.8, 81.2, 116, 165, 235, 336, 480, 686, 980, 1400 and 2000 μg/ml. Under these conditions, no cells were noted in cultures treated with 980 μg/ml and above. Cultures treated with 336, 480 and 686 μg/ml demonstrated an >85% reduction in the cell monolayer confluence. A reduction of dividing cells and ~55% reduction in the cell monolayer confluence were observed in the cultures treated with 235 μg/ml. A slight reduction in dividing cells and a ~30% reduction in the cell monolayer confluence were observed in the cultures treated with 165 μg/ml. There was an approximate 15% reduction in the cell monolayer confluence in cultures treated with 116 μg/ml tetracaine. The results of the sponsor's assessment of cytotoxicity are presented in the table below:

Table 1:
Assessment of Toxicity for Chromosome Aberrations Assay - Without Metabolic Activation - 3.0 Hour Treatment, 20.1 Hour Harvest

Assay No.: 23841 Trial No.: B1 Date: 06/13/02 Lab No.: CY7052
Test Article: Tetracaine Base

Treatment			Confluence* % Vehicle Control	% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	McCoy's 5a		100	10.8	11.4	11.1	---
Vehicle Control	DMSO	10.0 µL/mL	100	13.1	13.9	13.5	0
Test Article		81.2 µg/mL	100	15.3	15.6	15.5	0
		116 µg/mL	86	13.6	14.8	14.2	0
		165 µg/mL	71	13.2	13.9	13.6	0
		235 µg/mL	43	12.0	12.6	12.3	9

* This endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.
McCoy's 5a = culture medium DMSO = Dimethylsulfoxide

As noted above, reductions of 0%, 0%, 0% and 9% were observed in the mitotic indices of the cultures treated with 81.2, 116, 165 and 235 µg/ml. Chromosome aberrations were analyzed from cultures without metabolic activation treated with 81.2, 116, 165 and 235 µg/ml tetracaine. The concentration of 235 µg/ml tetracaine resulted in a 55% reduction in the cell monolayer confluence, and the concentration of 165 µg/ml produced a 49% reduction in mitotic index. The sponsor indicates that these observations demonstrate adequate toxicity for a valid high concentration for analysis. As these concentrations, there were no significant increases in cells with chromosome aberrations, polyploidy or endoreduplication in the cultures analyzed. The results are reproduced in sponsor's table 2 below:

23841-0-437OECD

Table 2:
Chromosome Aberrations in Chinese Hamster Ovary Cells - Without Metabolic Activation - 3.0 Hour Treatment, 20.1 Hour Harvest

Assay No.: 23841 Trial No.: B1 Date: 06/13/02 Lab No.: CY7052 Test Article: Tetracaine Base

			CELLS SCORED	% MITOTIC INDEX REDUC-TION[a]	# ENDO-REDUPLI-CATED CELLS	# POLY-PLOID CELLS	JUDGE-MENT (+/-)[b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS					TOTALS[c]		JUDGE-MENT (+/-)[d]
								Gaps	Simple Breaks	chte	chre	mab	-g	+g	
CONTROLS															
NEGATIVE: McCoy's 5a			A 100		0	0		2					0	2	
			B 100		0	0		2		1			1	3	
			TOTAL 200					4		1			1	5	
			AVERAGE %	-	0.0	0.0		2.0		0.5			0.5	2.5	
VEHICLE:	DMSO	10.0µL/mL	A 100		0	0		3					0	3	
			B 100		0	0		2					0	2	
			TOTAL 200					5					0	5	
			AVERAGE %	0	0.0	0.0		2.5					0.0	2.5	
POSITIVE:	MMC	0.750µg/mL	A 50		0	0		7	13	8	1	17	35	39	
			B 50		0	0		6	15	13		19	42	44	
			TOTAL 100					13	28	21	1	36	77	83	
			AVERAGE %	-	0.0	0.0	-	13.0	28.0	21.0	1.0	36.0	77.0	83.0	+
TEST ARTICLE		81.2µg/mL	A 100		0	0			2				2	2	
			B 100		0	0		3					0	3	
			TOTAL 200					3	2				2	5	
			AVERAGE %	0	0.0	0.0	-	1.5	1.0				1.0	2.5	-
		116µg/mL	A 100		1	0			2		1		3	3	
			B 100		1	0							0	0	
			TOTAL 200						2		1		3	3	
			AVERAGE %	0	1.0	0.0	-		1.0		0.5		1.5	1.5	-
		165µg/mL	A 100		3	0		3	1				1	4	
			B 100		2	0		2	1				1	3	
			TOTAL 200					5	2				2	7	
			AVERAGE %	0	2.5	0.0	-	2.5	1.0				1.0	3.5	-
		235µg/mL	A 100		3	0							0	0	
			B 100		2	0		2		1	2		3	5	
			TOTAL 200					2		1	2		3	5	
			AVERAGE %	9	2.5	0.0	-	1.0		0.5	1.0		1.5	2.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$.

McCoy's 5a = culture medium DMSO = Dimethylsulfoxide MMC = Mitomycin C

In a preliminary assay to determine dosing in the presence of with metabolic activation, precipitation was observed with culture concentrations of 980, 1400 and 2000 µg/ml and

above. No cell monolayer or dividing cells were observed in the cultures treated with 480, 686, 980, 1400 and 2000 µg/ml tetracaine. No dividing cells and a ~90% reduction in the cell monolayer confluence were observed in the cultures treated with 336 µg/ml. A reduction of the cell monolayer of ~55% was observed in cultures treated with 235 µg/ml. Reductions in mitotic indices of 18%, 41%, 49% and 36% were observed in cultures treated with 81.2, 116, 165 and 235 µg/ml. The results of the cytotoxicity assessment are presented in sponsor's table 3 below:

Table 3:
Assessment of Toxicity for Chromosome Aberrations Assay - With Metabolic Activation - 3.0 Hour Treatment, 20.1 Hour Harvest

Assay No.: 23841 Trial No.: B1 Date: 06/13/02 Lab No.: CY7052
Test Article: Tetracaine Base

Treatment			Confluence* % Vehicle Control	% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	McCoy's 5a		100	8.3	8.9	8.6	---
Vehicle Control	DMSO	10.0 µL/mL	100	8.4	8.6	8.5	0
Test Article		81.2 µg/mL	71	7.1	6.8	7.0	18
		116 µg/mL	86	3.9	6.0	5.0	41
		165 µg/mL	71	4.3	4.3	4.3	49
		235 µg/mL	43	4.8	5.9	5.4	36

* This endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.
McCoy's 5a = culture medium DMSO = Dimethylsulfoxide

Based upon these results, chromosome aberrations were analyzed from cultures treated with 81.2, 116, 165 and 235 µg/ml tetracaine.

Table 4:
Chromosome Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation – 3.0 Hour Treatment, 20.1 Hour Harvest

Assay No.: 23841 Trial No.: B1 Date: 06/13/02 Lab No.: CY7052 Test Article: Tetracaine Base

			CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS							JUDGEMENT (+/-) [d]
													TOTALS [c]		
CONTROLS								Gaps	Simple Breaks	chte	chre	mab	-g	+g	
NEGATIVE: McCoy's 5a		A	100		0	0							0	0	
		B	100		3	0					1		1	1	
		TOTAL	200								1		1	1	
		AVERAGE	%	-	1.5	0.0					0.5		0.5	0.5	
VEHICLE: DMSO	10.0µL/mL	A	100		0	0		1					0	1	
		B	100		0	0				1			1	1	
		TOTAL	200					1		1			1	2	
		AVERAGE	%	0	0.0	0.0		0.5		0.5			0.5	1.0	
POSITIVE: CP	5.00µg/mL	A	50		3	0		5	12	8	1		17	21	
		B	50		2	0		6	11	6	1		16	19	
		TOTAL	100					11	23	14	2		33	40	
		AVERAGE	%	-	2.5	0.0	–	11.0	23.0	14.0	2.0		33.0	40.0	+
TEST ARTICLE	81.2µg/mL	A	100		0	0		1			1		1	2	
		B	100		0	0		2					0	2	
		TOTAL	200					3			1		1	4	
		AVERAGE	%	18	0.0	0.0	–	1.5			0.5		0.5	2.0	–
	116µg/mL	A	100		0	0		1					0	1	
		B	100		2	1		1			1		1	2	
		TOTAL	200					2			1		1	3	
		AVERAGE	%	41	1.0	0.5	–	1.0			0.5		0.5	1.5	–
	165µg/mL	A	100		1	0		2					0	2	
		B	100		1	0		3			1		1	4	
		TOTAL	200					5			1		1	6	
		AVERAGE	%	49	1.0	0.0	–	2.5			0.5		0.5	3.0	–
	235µg/mL	A	100		2	0		3	2	1	1		3	5	
		B	100		3	1		2					0	2	
		TOTAL	200					5	2	1	1		3	7	
		AVERAGE	%	36	2.5	0.5	–	2.5	1.0	0.5	0.5		1.5	3.5	–

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

Negative controls: In non-activation assay, the negative controls were cultures containing only cells and culture medium. Vehicle controls were cultures containing the vehicle for tetracaine, DMSO at 10.0 μL/ml (the highest concentration of DMSO that was used in any test culture).

Positive controls: The positive control conditions are presented in the table below:

Assay Conditions	Treatment Duration	Positive Control	Concentration (μg/ml)
+ S9	3 hr	Cyclophosphamide (CP)	5 and 10
- S9	3 hr	Mitomycin C (MMC)	0.75 and 1.5
	20 hr		0.2 and 0.4

Incubation and sampling times: CHO cells were grown in McCoy's 5a culture medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin G (100 units/ml) and streptomycin (100 μg/ml) in a humidified incubator at 37°C in an atmosphere of 5% CO_2 in air. For the assay without S9, cultures were incubated in the presence of test article for 3 hours and 20 hours. For the assay with S9, cultures were incubated for 3 hours with test article, then washed with buffered saline, re-fed with new medium, and incubated for the remainder of the culture period. For the last 2 ± 0.5 hours of incubation, Colcemid® (0.1 μg/ml). Prior to harvest, cells were examined visually for evidence of cytotoxicity, percent confluence of the cell monolayer, presence of mitotic (large rounded cells) or dead cells floating in the medium. Cultures were then trypsonized for collection, swollen with 75 mM KCl, fixed in methanol:glacial acetic acid (3:1, v/v) fixative, stained with Giemsa solution for analysis of mitotic index and chromosomal aberrations.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears valid for the following reasons: 1) The positive controls cyclophosphamide and mitomycin C produced clear increases in chromosomal aberrations; 2) the assay was conducted according to standard protocols, 3) the concentrations tested were adequate to produce a valid assay, and 4) the negative controls produced an acceptable level of chromosomal aberrations.

Study outcome: In the confirmatory assay without metabolic activation, tetracaine concentrations of 300 and 350 μg/ml produced > 85% reduction in the cell culture monolayer confluence, dead cells, debris and no dividing cells. Concentration of 250 μg/ml resulted in ~70% reduction in the cell monolayer confluence, dead cells and a reduction in dividing cells. The concentration of 200 μg/ml tetracaine produced unhealthy cell monolayers with ~45% reduction in dividing cells. sponsor's Table 5, reproduced below, indicates that concentrations of tetracaine of 75 mg/ml and above produced significant toxicity (> 50%) as measured by a reduction in the mitotic index.

Table 5:
Assessment of Toxicity for Chromosome Aberrations Assay - Without Metabolic Activation - 19.8 Hour Treatment, 19.8 Hour Harvest

Assay No.: 23841 Trial No.: C1 Date: 07/02/02 Lab No.: CY8052
Test Article: Tetracaine Base

Treatment			Confluence* % Vehicle Control	% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	McCoy's 5a		100	9.7	10.6	10.2	---
Vehicle Control	DMSO	10.0 µL/mL	100	9.9	9.8	9.9	0
Test Article		9.40 µg/mL	100	8.3	8.8	8.6	13
		18.8 µg/mL	100	6.3	7.4	6.9	30
		37.5 µg/mL	100	6.2	6.7	6.5	34
		75.0 µg/mL	100	3.9	5.0	4.5	55
		150 µg/mL	100	3.7	4.1	3.9	61
		200 µg/mL	57	1.8	2.4	2.1	79
		250 µg/mL	29	0.0	0.2	0.1	99
		300 µg/mL	14	0.0	0.0	0.0	100

* This endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.
McCoy's 5a = culture medium DMSO = Dimethylsulfoxide

Based upon the cytotoxicity results above, chromosomal aberrations from cultures treated with 9.4, 18.8, 37.5 and 75 µg/ml tetracaine were scored. As shown in sponsor's table 8 below, there were no significant increases in chromosomal aberrations, polyploidy or endoreduplication noted in these cultures.

Additional cytotoxicity assessments were made for the definitive assay in the presence of metabolic activation. As depicted in sponsor's table 7 below, a concentration of 300 µg/ml tetracaine produced a 43% reduction in mitotic index but an ~ 85% reduction in cell monolayer confluence. In contrast, only slight evidence of cytotoxicity was noted at the next highest concentration tested (250 µg/ml). In the definitive 3 hour treatment of CHO cells in the presence of metabolic activation are presented in the sponsor's Table 8 depicted below. The results indicate that a significant increase in cells with chromosomal aberrations was observed in the cultures treated with 300 mg/ml. There was also a significant increase in endoreduplication observed in cultures treated with 250 and 300 µg/ml tetracaine.

Table 7:
Assessment of Toxicity for Chromosome Aberrations Assay - With Metabolic Activation - 3.0 Hour Treatment, 19.8 Hour Harvest

Assay No.: 23841 Trial No.: C1 Date: 07/02/02 Lab No.: CY8052
Test Article: Tetracaine Base

Treatment			Confluence[a] % Vehicle Control	% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	McCoy's 5a		100	11.1	11.5	11.3	---
Vehicle Control	DMSO	10.0 µL/mL	100	10.2	11.5	10.9	0
Test Article		150 µg/mL	100	11.6	12.8	12.2	0
		200 µg/mL	100	9.6	10.2	9.9	9
		250 µg/mL	86	7.2	8.5	7.9	28
		300 µg/mL	14	5.9	6.4	6.2	43

[a] This endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.
McCoy's 5a = culture medium DMSO = Dimethylsulfoxide

As noted in sponsor's Table 6 and 8 below, there were no significant increases in chromosomal abnormalities following a 3 or 20 hour exposure to lidocaine in the absence of metabolic activation or 3 hour exposure to lidocaine in the presence of metabolic activation, respectively.

Table 6:
Chromosome Aberrations in Chinese Hamster Ovary Cells - Without Metabolic Activation – 19.8 Hour Treatment, 19.8 Hour Harvest

Assay No.: 23841 Trial No.: C1 Date: 07/02/02 Lab No.: CY8052 Test Article: Tetracaine Base

			CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS							JUDGEMENT (+/-) [d]
								Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	
CONTROLS															
NEGATIVE: McCoy's 5a		A	100		0	2		5					0	5	
		B	100		0	3		6	1		2		3	9	
		TOTAL	200					11	1		2		3	14	
		AVERAGE	%	-	0.0	2.5		5.5	0.5		1.0		1.5	7.0	
VEHICLE: DMSO	10.0µL/mL	A	100		0	3		10	4				4	12	
		B	100		0	2		13	4	1	1		6	18	
		TOTAL	200					23	8	1	1		10	30	
		AVERAGE	%	0	0.0	2.5		11.5	4.0	0.5	0.5		5.0	15.0	
POSITIVE: MMC	0.200µg/mL	A	50		0	3		10	16	11		3	23	29	
		B	50		2	3		9	15	11	3	3	27	32	
		TOTAL	100					19	31	22	3	6	50	61	
		AVERAGE	%	-	1.0	3.0	-	19.0	31.0	22.0	3.0	6.0	50.0	61.0	+
TEST ARTICLE	9.40µg/mL	A	100		0	4		7	1	1			2	9	
		B	100		0	2		12					0	12	
		TOTAL	200					19	1	1			2	21	
		AVERAGE	%	13	0.0	3.0	-	9.5	0.5	0.5			1.0	10.5	-
	18.8µg/mL	A	100		0	2		6	2				2	8	
		B	100		0	2		4	2				2	6	
		TOTAL	200					10	4				4	14	
		AVERAGE	%	30	0.0	2.0	-	5.0	2.0				2.0	7.0	-
	37.5µg/mL	A	100		0	2		8	2				3	10	
		B	100		0	3		5	1		1		3	8	
		TOTAL	200					13	3		1	1	6	18	
		AVERAGE	%	34	0.0	2.5	-	6.5	1.5		2	1	3.0	9.0	-
	75.0µg/mL	A	100		0	2		6			1.0	0.5	0	6	
		B	100		0	3		4					0	4	
		TOTAL	200					10					0	10	
		AVERAGE	%	55	0.0	2.5	-	5.0					0.0	5.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = Dimethylsulfoxide MMC = Mitomycin C

Table 8:
Chromosome Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation – 3.0 Hour Treatment, 19.8 Hour Harvest

Assay No.: 23841 Trial No.: C1 Date: 07/02/02 Lab No.: CY8052 Test Article: Tetracaine Base

CONTROLS		CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS							JUDGEMENT (+/-) [d]
							Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	
NEGATIVE: McCoy's 5a		A 100		0	2		2					0	2	
		B 100		0	4		6	1				1	7	
		TOTAL 200					8	1				1	9	
		AVERAGE %	-	0.0	3.0		4.0	0.5				0.5	4.5	
VEHICLE: DMSO	10.0µL/mL	A 100		1	3		3					0	3	
		B 100		0	2		5	1				1	5	
		TOTAL 200					8	1				1	8	
		AVERAGE %	0	0.5	2.5		4.0	0.5				0.5	4.0	
POSITIVE: CP	5.00µg/mL	A 50		0	2		5	17	8	2		23	26	
		B 50		0	3		3	18	8	2	4	27	29	
		TOTAL 100					8	35	16	4	4	50	55	
		AVERAGE %	-	0.0	2.5	–	8.0	35.0	16.0	4.0	4.0	50.0	55.0	+
TEST ARTICLE	150µg/mL	A 100		2	4		5	1				1	6	
		B 100		2	3		7	2		2		4	10	
		TOTAL 200					12	3		2		5	16	
		AVERAGE %	0	2.0	3.5	–	6.0	1.5		1.0		2.5	8.0	–
	200µg/mL	A 100		1	2		7	1				1	8	
		B 100		1	4		4					1	5	
		TOTAL 200					11	1		1		2	13	
		AVERAGE %	9	1.0	3.0	–	5.5	0.5		0.5		1.0	6.5	–
	250µg/mL	A 100		8	4		9	3	1			4	13	
		B 100		7	3		3	2				2	5	
		TOTAL 200					12	5	1			6	18	
		AVERAGE %	28	7.5	3.5	+	6.0	2.5	0.5			3.0	9.0	–
	300µg/mL	A 100		13	5		17	13	4			16	31	
		B 100		15	5		9	8	4			12	20	
		TOTAL 200					26	21	8			28	51	
		AVERAGE %	43	14.0	5.0	+	13.0	10.5	4.0			14.0	25.5	+

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$.

McCoy's 5a = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

Study title: ***In Vivo*** **Mouse Micronucleus Assay with Lidocaine Base**

Key findings: Under the conditions of the assay, lidocaine base was negative in the *in vivo* mouse bone micronucleus assay. However, the dose concentration samples could not be analyzed due to technical difficulties and therefore not verified. Given the toxicity noted, this does not jeopardize the conclusions of the study.

Study no.: 23840-0-455OECD
Volume #, and page #: Volume 12, Page 133
Conducting laboratory and location: [redacted]

Date of study initiation: May 13, 2002
GLP compliance: Yes
QA reports: yes (X) no ( ). NOTE. QA report indicates that a critical portion of the study was not audited by the Quality Assurance auditor. This deviation was determined to have no impact on the integrity of the study or the interpretation of the results.
Drug, lot #, and % purity: Lidocaine base, Lot No. 811D0013, not indicated. Test article samples were sent to [redacted] for analysis; however, they were not able to be analyzed due to "technical difficulties."

Methods

Strains/species/cell line: :CD-1 (ICR) BR Mouse.

Doses used in definitive study: 0, 50, 100 and 200 mg/kg, i.p. as summarized in the following table (sponsor's):

Dosing Scheme for the Micronucleus Assay with Lidocaine Base

Target Treatment (mg/kg)	Stock Concentration (mg/mL)	Route of Administration	Dosing Volume (mL/kg)	Animals/Harvest Timepoint[a] 24 Hour Male	48 Hour Male	Replacement Animals[b] Male
50	2.5	intraperitoneal injection	20	6	-	-
100	5.0	intraperitoneal injection	20	6	-	-
200	10.0	intraperitoneal injection	20	6	6	6
Vehicle Control, cell culture grade water	0	intraperitoneal injection	20	6	6	-
Positive Control, Cyclophosphamide, 80	8	oral gavage	10	6	-	-

[a] Six animals were dosed to ensure the availability of five animals for analysis.
[b] Animals were dosed as potential replacements for the original high-dose groups.
Note: Animals not used as replacements were euthanized at the completion of the trial.

Basis of dose selection: A dose range finding study was conducted with both males and females. No doses higher than 200 mg/kg were tested due to solubility constraints. The

maximum tolerated dose was determined from daily observations of toxic signs and/or mortality. There were no relevant differences in toxicity noted between males and females, therefore only males were used in the micronucleus assay (this is acceptable based upon current methodology).

In the dose range-finding study, lidocaine doses of 100 mg/kg and 200 mg/kg (i.p.) were administered to 3 mice/sex in a dosing volume of 20 mg/kg. Lidocaine doses were prepared prior to dosing. Animals were observed immediately after dosing, approximately 1 hour after dosing and daily for the duration of the assay for toxic signs and/or mortality. There were no mortalities in this study. The toxicities noted all occurred immediately post dosing and are summarized in the table below:

Incidence of Clinical Signs in Dose Range Finding Study Immediately Post-Dosing				
Toxicity (N=3/sex)	100 mg/kg		200 mg/kg	
	Males	Females	Males	Females
Flattened posture	1	2	3	1
Labored respiration	1	2	2	0
Ataxia	0	2	3	3
Convulsions	0	2	0	0

From the above results, the sponsor concluded that a dose of 200 mg/kg was determined to be the maximum tolerated dose. The above table indicates that lidocaine treatment produced convulsions in 2 of 3 females following administration of 100 mg/kg but not 200 mg/kg. Convulsions are clearly a sign of a maximum tolerated dose and they can frequently lead to death, however the lack of a dose-dependency of this finding and differential effects between males and females makes the sponsor's conclusions difficult to defend based exclusively on these findings (see definitive study results below).

Negative controls: The vehicle control article was cell culture grade water.

Positive controls: Cyclophosphamide (CP from [redacted] 80 mg/kg via oral gavage. CP was dissolved in cell culture grade water.

Incubation and sampling times: Following treatment, animals were sacrificed at 24 hours. Animal observations were conducted as described for the dose range finding study. Bone marrow was extracted at either 24 or 48 hours post dosing via flushing with fetal bovine serum. Slides were prepared and the cells were fixed in methanol, stained with May-Grünwald solution followed by Geimsa for analysis. Slides from the first 5 animals per group were scored for micronuclei and the polychromatic and normochromatic erythrocyte ratio was determined. Micronuclei frequency was determined by analyzing the number of micronucleated PCE format least 200 PCEs per animal. The PCE:NCE ratio was determined by scoring PCEs and NCEs observed while scoring at least the first 500 erythrocytes per animal. The historical background for the frequency of micronuclei in the CD-1 strain at [redacted] laboratory is about 0.0 to 0.4%.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The sponsor described their acceptance criteria as follows:

> **Acceptable Controls:** The vehicle control group had less than approximately 0.4% micronucleated PCEs and the group mean was within the historical control range. The positive control group had a statistically significantly higher ($p < 0.01$) number of micronucleated PCEs than the vehicle control group and was consistent with historical positive control data.
> **Acceptable High Dose:** The high dose produced clinical signs of toxicity and mortality in the animals.

The study appears to valid for the following reasons: 1) the positive control, cyclophosphamide, produced a clear response, 2) the number of cells scored per animal is within acceptable protocols, 3) the dosing reached an MTD as defined by mortality and convulsions, 4) the background data is within the historical control range of <0.4%.

Study outcome: The doses used in the definitive study produced acceptable toxicity to establish a maximum tolerated dose. The clinical signs are summarized in the sponsor's table below:

Animal Observations for Toxicity for Micronucleus Assay with Lidocaine Base

Target Dose Level (mg/kg)	Harvest Timepoint	Animal ID	Time After Dosing			
			IPD	1 hour PD	1 day	2 days
50	24	3394	1,2,3	0	0	NA
		3403	1,2,3	0	0	NA
		3409	1,2,3	0	0	NA
		3420	1,2,3	0	0	NA
		3422	1,2,3	0	0	NA
		3429	1,2,3	0	0	NA
100	24	3389	1,2,3	0	0	NA
		3392	1,2,3	0	0	NA
		3396	1,2,3	0	0	NA
		3401	1,2,3	0	0	NA
		3419	1,2,3	0	0	NA
		3426	1,2,3	0	0	NA
200	24	3386	1,2,3	9	-	-
		3406	1,2,3	4,10,11	0	NA
		3412	1,2,3	4,10,11	0	NA
		3416	2,4	0	0	NA
		3428	2,4	4,10,11	0	NA
		3432	2,4	9	-	-
	48	3387	1,2,4,8	4,10	0	0
		3388	6→5	-	-	-
		3397	2,4,8	4,10	0	0
		3400	2,4,8	4,10	0	0
		3417	2,4,7	4,10	0	0
		3427	6→5	-	-	-
	Secondary	3393	1,2,3	0	0	NA
		3414	2,4,8	1,3	0	0
		3423	1,2,3	0	0	0
		3425	2,4,8	1,3	0	0
		3430	2,4,5	-	-	-
		3431	2,4,8	9	-	-

Key: 0 = Normal, 1 = Ataxic, 2 = flattened posture, 3 = slightly hypoactive, 4 = hypoactive, 5 = died approximately 5 minutes postdose, 6 = convulsions, 7 = gasping, 8 = labored breathing, 9 = found dead, 10 = hunched posture, 11 = squinted eyes; →followed by
IPD = immediately post dosing last animal, PD = post dosing last animal, NA = not applicable, animal harvested at the 24-hour harvest timepoint.

As indicated in the table above, one of 6 animals in the high dose group were found dead approximately 1 hour post dosing. Two animals in the 48 hour group had convulsions which preceded death, indicating that **the maximum tolerated dose was achieved** in this study.

The results of the micronucleus assay are reproduced in sponsor's table 1 below. As indicated in the table, cyclophosphamide produced a significant increase in the % micronucleated PCEs. In contrast, lidocaine doses of up to 200 mg/kg did not produce an increase in % micronucleated PCEs at either 24 or 48 hour time points. The lack of an effect on the PCE:NCE ratio indicates a lack of bone marrow toxicity in this study.

Table 1: Micronucleus Data Summary Table

ASSAY NO.: 23840
TEST ARTICLE: Lidocaine Base

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E. MALES	RATIO PCE:NCE MEAN ± S.E. MALES
CONTROLS				
VEHICLE	CCGW	24 hr	0.05 ± 0.00	0.59 ± 0.02
		48 hr	0.10 ± 0.02	0.50 ± 0.05
POSITIVE	CP 80 mg/kg	24 hr	3.63 ± 0.37*	0.52 ± 0.03
TEST ARTICLE	50 mg/kg	24 hr	0.04 ± 0.04	0.51 ± 0.05
	100 mg/kg	24 hr	0.07 ± 0.03	0.57 ± 0.06
	200 mg/kg	24 hr	0.06 ± 0.01	0.58 ± 0.09
		48 hr	0.05 ± 0.00	0.65 ± 0.05

* Significantly greater than the corresponding vehicle control, $p<0.01$.
CCGW = Cell culture grade water
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Study title: ***In Vivo*** **Mouse Micronucleus Assay with Tetracaine Base**

Key findings: Under the conditions tested, tetracaine base was negative in the *in vivo* mouse bone marrow micronucleus assay.

Study no.: 23841-0-455OECD
Volume #, and page #: Volume 12, Page 154
Conducting laboratory and location: [redacted]
Date of study initiation: May 13, 2002
GLP compliance: Yes
QA reports: yes (X) no ( ) NOTE: there is no statement as to any portions of the study that were not subject to quality assurance inspections.
Drug, lot #, and % purity: Tetracaine base, Lot No. 721725, Samples were shipped to [redacted] for analysis. Upon receipt at [redacted] it was determined that the samples could not be analyzed due to technical difficulties.

Methods

Strains/species/cell line: :CD-1 (ICR) BR mouse model.

Doses used in definitive study: 0, 125, 250 and 500 mg/kg tetracaine.

Basis of dose selection: A dose-range-finding study was conducted in both males and females (n=up to 3/sex/group). Doses higher than 2000 mg/kg were not tested based on information provided by the sponsor. Daily observations of toxicity or mortality were used to estimate the maximum tolerated dose. There were no relevant differences in toxicity noted between males and females, therefore only males were used in the micronucleus assay (this is acceptable based upon current methodology).

In the dose range-finding study, tetracaine doses of 1000 mg/kg, 1500 mg/kg and 2000 mg/kg (i.p.) were administered to up to 3 mice/sex/group in a dosing volume of 20 mg/kg. Tetracaine doses were prepared prior to dosing. Animals were observed immediately after dosing, approximately 1 hour after dosing and daily for the duration of the assay for toxic signs and/or mortality. The toxicities noted all occurred immediately post dosing and are summarized in the tables below:

Summary of Mortalities for Dose Rangefinding Assay with Tetracaine Base

Target Treatment (mg/kg)	Number of Males (Died/Total Dosed)	Number of Females (Died/Total Dosed)
1000	0/3[a]	0/3
1500	2/3	1/3
2000	1[b]/1	1/1

[a]One animal's death may have been due to a possible dosing complication; the death is therefore deemed to have been accidental.

[b]The male was humanely sacrificed per Study Director.

As noted above, 1 of 3 females and 2 of 3 males treated with 1500 mg/kg died following dosing. In addition 1 of 1 females dosed treated with 2000 mg/kg tetracaine died following clinical signs of tremors and recumbency (see table below for clinical signs). The single male treated with 2000 mg/kg demonstrated clinical signs of ataxia, labored respiration and flattened posture and was sacrificed within 1 hour of treatment *in extremis*.

Animal Observations for Toxicity for Dose Rangefinding Assay with Tetracaine Base

Target Dose Level (mg/kg)	Sex	Animal ID	Time After Dosing			
			IPD	1 hour PD	1 day	2 days
1000	M	2984	0	0	0	0
		2987	1[a]	-	-	-
		2990	2,3	4	0	0
	F	2994	0→2,8	0	0	0
		2996	3	0	0	0
		2998	3	0	0	0
1500	M	2985	2,3	0	0	0
		3003	1	-	-	-
		3004	1	-	-	-
	F	2999	5,6,7	0	0	0
		3000	0	0	0	0
		3001	1	-	-	-
2000	M	2986	0→3	5,6,7→9	-	-
	F	2993	2,3→1	-	-	-

Key: 0 = Normal, 1 =found dead, 2 = tremors, 3 = recumbent, 4 = slightly hypoactive, 5 = ataxic, 6 = labored respiration, 7 = flattened posture, 8 = animal on its back, 9 = humanely sacrificed per Study Director
IPD = immediately post dosing last animal, PD = post dosing last animal, → = followed by
[a]Death may have been due to a possible dosing complication; the death is therefore deemed to have been accidental.

From the above results, the sponsor concluded that a dose of 1000 mg/kg tetracaine was determined to be the maximum tolerated dose. Initial dosing for the micronucleus assay was to be 250, 500 and 1000 mg/kg, however, due to excessive mortality in the 1000 mg/kg group as well as a 750 mg/kg preliminary dosing group; the high dose was set at 500 mg/kg. The final dosing design is presented below:

Dosing Scheme for the Micronucleus Assay with Tetracaine Base

Target Treatment (mg/kg)	Stock Concentration (mg/mL)	Route of Administration	Dosing Volume (mL/kg)	Animals/Harvest Timepoint[a]	
				24 Hour Male	48 Hour Male
125	6.25	intraperitoneal injection	20	7	-
250	12.5	intraperitoneal injection	20	6	-
500	25.0	intraperitoneal injection	20	6	6
Vehicle Control, cell culture grade water	0	intraperitoneal injection	20	6	6
Positive Control, Cyclophosphamide, 80	8	oral gavage	10	6	-

[a] Six animals were dosed to ensure the availability of five animals for analysis.
[b] Animals were dosed as potential replacements for the original high-dose groups.
Note: Animals not used as replacements were euthanized at the completion of the trial.

Negative controls: The vehicle control article was cell culture grade water.

Positive controls: Cyclophosphamide (CP from [redacted] served as the positive control (80 mg/kg oral gavage) and was dissolved in cell culture grade water.

Incubation and sampling times: Following treatment, animals were sacrificed at 24 hours. Animal observations were conducted as described for the dose range finding study. Bone marrow was extracted at either 24 or 48 hours post dosing via flushing with fetal bovine serum. Slides were prepared and the cells were fixed in methanol, stained with May-Grünwald solution followed by Geimsa for analysis. Slides from the first 5 animals per group were scored for micronuclei and the polychromatic and normochromatic erythrocyte ratio was determined. Micronuclei frequency was determined by analyzing the number of micronucleated PCE format least 200 PCEs per animal. The PCE:NCE ratio was determined by scoring PCEs and NCEs observed while scoring at least the first 500 erythrocytes per animal. The historical background for the frequency of micronuclei in the CD-1 strain at [redacted] laboratory is about 0.0 to 0.4%.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The sponsor described their acceptance criteria as follows:

> **Acceptable Controls:** The vehicle control group had less than approximately 0.4% micronucleated PCEs and the group mean was within the historical control range. The positive control group had a statistically significantly higher ($p < 0.01$) number of micronucleated PCEs than the vehicle control group and was consistent with historical positive control data.
> **Acceptable High Dose:** The high dose produced clinical signs of toxicity and mortality in the animals.

The study appears to valid for the following reasons: 1) the positive control, cyclophosphamide, produced a clear response, 2) the number of cells scored per animal is within acceptable protocols, 3) the dosing reached an MTD as defined by mortality and convulsions, 4) the background data is within the historical control range of <0.4%.

Study outcome: There were no mortalities noted following treatment with 125, 250 or 500 mg/kg tetracaine. As noted in the table below, most animals in the 250 and 500 mg/kg groups demonstrated clinical signs of ataxia, irregular respiration, flattened posture, hypoactivity and head tremors immediately post dosing.

Animal Observations for Toxicity for Micronucleus Assay with Tetracaine Base

Target Dose Level (mg/kg)	Harvest Timepoint	Animal ID	Time After Dosing			
			IPD	1 hour PD	1 day	2 days
125	24	3458	2,5,6	0	0	NA
		3460	0	0	0	NA
		3461	0	0	0	NA
		3462	0	0	0	NA
		3463	0	0	0	NA
		3464	0	0	0	NA
		3465	0→1	0	0	NA
250	24	3341	1,2,3,4	0	0	NA
		3351	0	0	0	NA
		3352	1,2,3,4	0	0	NA
		3356	1,2,3,4	0	0	NA
		3379	1,2,3,4	0	0	NA
		3381	1,2,3,4	0	0	NA
500	24	3340	1,2,3,4,5	0	0	NA
		3346	1,2,3,4,5	0	0	NA
		3347	1,2,3,4,5	0	0	NA
		3354	1,2,3,4,5	0	0	NA
		3366	1,2,3,4,5	0	0	NA
		3374	0	0	0	NA
	48	3350	0	0	0	0
		3353	0	0	0	0
		3358	0	0	0	0
		3378	0	0	0	0
		3382	0	0	0	0
		3383	0	0	0	0

Key: 0 = Normal, 1 = ataxic, 2 = irregular respiration, 3 = flattened posture, 4 = hypoactive, 5 = head tremors, 6 = recumbent, IPD = immediately post dosing last animal, PD = post dosing last animal, NA = not applicable, animal harvested at the 24-hour harvest timepoint.

The summary of the micronucleus data is reproduced in the sponsor's table below. Although cyclophosphamide produced a clear increase in the % micronucleated PCE, tetracaine base tested negative under the conditions of this assay.

Table 1: Micronucleus Data Summary Table

ASSAY NO.: 23841
TEST ARTICLE: Tetracaine Base

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E. MALES	RATIO PCE:NCE MEAN ± S.E. MALES
CONTROLS				
VEHICLE	Water	24 hr	0.10 ± 0.02	0.40 ± 0.04
		48 hr	0.02 ± 0.01	0.58 ± 0.06
POSITIVE	CP 80 mg/kg	24 hr	3.38 ± 0.48*	0.50 ± 0.04
TEST ARTICLE	125 mg/kg	24 hr	0.09 ± 0.05	0.53 ± 0.04
	250 mg/kg	24 hr	0.10 ± 0.02	0.68 ± 0.07
	500 mg/kg	24 hr	0.03 ± 0.01	0.57 ± 0.05
		48 hr	0.05 ± 0.03	0.48 ± 0.04

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

3.4.5. Carcinogenicity

Studies examining the carcinogenic potential of either lidocaine or tetracaine have not been completed. These studies are not required for drug products which are not used on a chronic basis, as described in ICH M3 Guidance.

The metabolite of lidocaine 2,6-xylidine, has been tested for carcinogenic potential in Sprague-Dawley rats by the National Toxicology Program in 1990. Dietary administration of 2,6-xylidine at 0, 300, 1000 or 3000 ppm for 102 weeks produced a significant increase in the incidence of nasal cavity adenomas, carcinomas and adenocarcinomas in both males and females. Rhabdomyosarcomas, previously unreported in this rat strain, were detected in two high-dose males and two high-dose females (none in the controls) (National Toxicology Program, 1990).

As previously detailed by Dr. McGovern in the first cycle NDA review of NDA 21-431, The major lidocaine metabolite 2,6-xylidine is "carcinogenic in mice and/or rats (see the reviews for NDA 19-941 dated June 19, 1992 and September 28, 1992 by Dr. Dou Lucy Jean for details). 2,6-xylidine produced carcinomas and adenomas in the nasal cavity and a rhabdomyosarcoma (rare) in rats at an oral dose of 150 mg/kg. An increased incidence of subcutaneous fibromas and/or fibrosarcomas was also noted in males while neoplastic nodules of the liver were observed in females. In May 1996, the relevancy of the rat tumor findings to humans was discussed by CDER's Executive Carcinogenicity Assessment Committee (see minutes dated May 14, 1996 under NDA 19-941).

The committee concluded that the tumor findings are not relevant to humans and should not be included in the labeling nor should the labeling of other anesthetic products containing lidocaine be revised."

3.4.6. Reproductive and developmental toxicology

A Segment I study for tetracaine was not submitted with this IND. The Division previously agreed to allow the Segment I study to be submitted post –approval (phase 4 commitment). The sponsor is relying upon published literature and previous findings of safety and efficacy to support the effects of lidocaine on fertility and early embryonic development.

Embryofetal development

Study title: Pilot Study for Effects on Embryo-Fetal Development in Rats (with Toxicokinetics)

Key study findings: A pilot study to determine dose levels for the definitive rat segment II study was conducted with a toxicokinetic portion was included. The key findings are as follows:

1. Under the conditions of the assay, no external variations or malformations were observed at any dose of Lidocaine or tetracaine tested.

Study no.: 925-012
Volume #, and page #: Volume 3, Page 1
Conducting laboratory and location: [redacted]
Date of study initiation: January 8, 2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Lidocaine base, Lot 811D0013, Tetracaine base, Batch 721724, [redacted]

Methods

Doses: Lidocaine doses were 15, 30, 60 and 75 mg/kg, s.c. Tetracaine doses were 1, 2, 5 and 10 mg/kg, s.c.
Species/strain: Sprague-Dawley female rats [[redacted] CD(SD)IGS BR]
Number/sex/group: 7 group (females)
Route, formulation, volume, and infusion rate: subcutaneous, vehicle was sodium phosphate buffer, volume was 1 ml/kg.
Satellite groups used for toxicokinetics: 7 rats/group
Study design: Pregnant rats were treated on gestation days 6-17 of gestation. Test article was administered to the main study animals and TK animals once a day.

Injections were made on the back of the animal in the scapular and lumbar regions, alternating left and right sides per day.

Group Assignment		
Group Number	Dose Level (mg/kg/day)	Number of Time-mated Female Rats
1	0 (Vehicle Control)	5
2	15 (Lidocaine)	5
3	30 (Lidocaine)	5
4	60 (Lidocaine)	5
5	75 (Lidocaine)	5
6	1 (Tetracaine)	5
7	2 (Tetracaine)	5
8	5 (Tetracaine)	5
9	10 (Tetracaine)	5
10[a]	15 (Lidocaine)	7
11[a]	30 (Lidocaine)	7
12[a]	60 (Lidocaine)	7
13[a]	75 (Lidocaine)	7
14[a]	1 (Tetracaine)	7
15[a]	2 (Tetracaine)	7
16[a]	5 (Tetracaine)	7
17[a]	10 (Tetracaine)	7

[a]Animals in Groups 10 through 17 were used for toxicokinetic evaluations

Parameters and endpoints evaluated:

Mortality and Cage-side Observations: twice daily. Detailed clinical examinations were conducted from Day 6 through 20 of gestation.

Body Weight and Body Weight Changes: Individual body weights for main study animals were recorded on Days 0, 6, 9, 12, 15, 18 and 20 of gestation. Individual body weight change for main study animals was calculated for the following gestation day intervals: 0-6, 6-9, 9-12, 12-15, 15-18, 18-20, 6-18 and 0-20. Adjusted body weight (Day 20 gestation body weight minus the gravid uterine weight) and adjusted body weight change (Days 0-20 of gestation) for main study animals were also calculated.

Food Consumption: Main study animal food consumption was recorded on corresponding body weight days and calculated for the following intervals: 0-6, 6-9, 9-12, 12-15, 15-18, 18-20, 6-18 and 0-20.

Toxicokinetics: Blood samples were collected from the orbital sinus after carbon dioxide/oxygen inhalation of six rats/TK group on Day 17 of gestation. Samples were collected at 0.5, 1, 2, 4, 8 and 24 hours post dose. Samples were placed in tubes containing potassium EDTA and neostigmine and stored on ice until centrifuged to collect the plasma. Plasma samples were stored at -70C until shipped for analysis. Blood samples collected for determination of plasma concentration of Tetracaine were not analyzed.

Maternal Necropsy: Complete necropsy was performed on all main study dams. Emphasis was placed on structural abnormalities or pathologic changes that

may have been influenced by the pregnancy. Gross lesions were saved in 10% neutral buffered formalin for possible microscopic examination. Following determination of the pregnancy status of the animal, the carcass was discarded without further analysis.

Ovarian and Uterine Examinations: On Day 20, main study dams were euthanized by carbon dioxide inhalation and the uterus and ovaries were exposed by a mid-abdominal incision. The location of viable and nonviable fetuses, early and late resorptions for each uterine horn, position of the cervix, and the total number of implantations were recorded. The number of corpora lutea on each ovary was recorded. The uterus was excised and gravid uterine weight recorded. Fetuses were removed, and the placenta was grossly examined. Fetuses were individually weighed, sexed externally, and examined for external malformations and variations. No malformations or variations were noted on external examination and all fetuses were euthanized and discarded.

Results

Mortality (dams): One pregnant rat in the 75 mg/kg/day lidocaine treatment group was found dead on Day 14 of gestation. There were no clinical signs leading to the death of the animal. There were no other deaths.

Clinical signs (dams): Clinical signs in dams during gestation are reviewed in the table below (extracted from the sponsor's table with only representative skin description noted). Overall, the highest dose of lidocaine and tetracaine increased the incidence of decreased activity and prostration. All other doses did not demonstrate this response. The skin changes were primarily described as regions showing only sparse hair or scabbed areas at the sights of injection. These were noted in lidocaine treated rats treated with 30 mg/kg lidocaine and above but not in the tetracaine treated rats at any dose tested. The observation of rapid breathing rate was observed in one rat treated with the 75 mg/kg dose of lidocaine or 5 animals treated with the 10 mg/kg dose of tetracaine demonstrated a rapid rate of respiration on several occasions.

Summary of Clinical Observations in the Dams (# times observed/total number of animals affected)

Group	Vehicle	Lidocaine				Tetracaine			
N	5	5	5	5	5	5	5	5	5
Dose (mg/kg)	0	15	30	60	75	1	2	5	10
Behavior									
Activity Decreased	0	1/1	0	0	10/1	0	0	0	67/5
Prostration	0	1/1	0	0	5/1	0	0	0	38/5
Skin									
Hair sparse, lumbar	0	0	0	13/1	6/1	0	0	0	0
Scabbed area, lumbar	0	0	12/1	54/5	40/5	0	0	0	0
Scabbed area, shoulder right	0	0	6/2	18/2	18/4	0	0	0	0
Scabbed area, shoulder left	0	0	8/2	29/4	41/5	0	0	0	0
Respiration									
Rapid breathing	0	2/1	0	0	6/1	0	0	0	55/5

Body weight (dams): There were no statistically significant changes in body weight noted following either lidocaine or tetracaine at any dose tested. There were no statistically significant differences in body weight change values between groups.

Food consumption (dams): Food consumption was not significantly altered by treatment with either lidocaine or tetracaine under the conditions of this assay.

Toxicokinetics: Figure 1 below depicts the mean lidocaine concentration for female rats on Day 17 of gestation. sponsor's table 1 below provides the mean plasma concentrations of lidocaine in female rats on Day 17 of gestation. The lower limit of quantitation of the assay was 0.9 ng/ml. The greatest concentration of lidocaine was noted at the first time point examined, 0.5 h. C_{max} increased with dose; however this increase was not proportional to the dose given. The concentration following 60 mg/kg was only slightly below that following 75 mg/kg. The t½ of the 15 mg/kg dose of lidocaine was 0.8 h, while the doses of 30, 60 and 75 mg/kg were 2.0, 2.4 and 2.4 respectively.

Figure 1. Mean Lidocaine Concentrations for Female Rats on Day 17 of Gestation (linear plot)

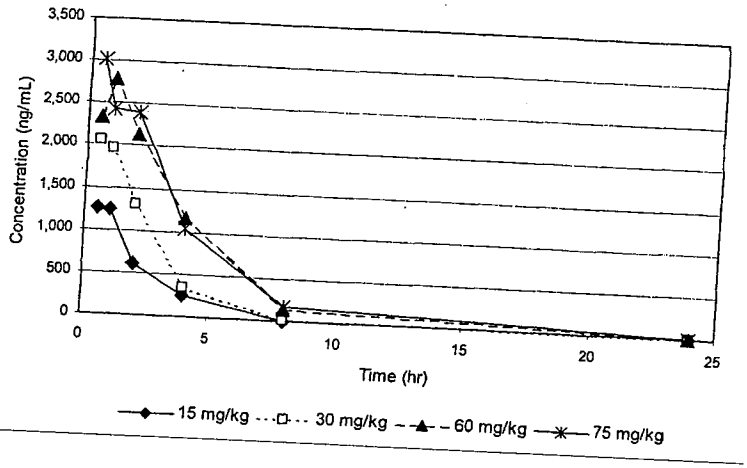

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Table 1. Mean Plasma Concentrations of Lidocaine in Female Rats on Day 17 of Gestation

Time (hr)	Mean Lidocaine Concentration (ng/mL)			
	15 mg/kg Mean ± SD	30 mg/kg Mean ± SD	60 mg/kg Mean ± SD	75 mg/kg Mean ± SD
0.5	1,267 ± 263	2,060 ± 104	2,336 ± 281	3,019 ± 1,078
1	1,249 ± 177	1,966 ± 54	2,788 ± 504	2,430 ± 554
2	614 ± 135	1,305 ± 333	2,128 ± 271	2,397 ± 82
4	248 ± 106	349 ± 98	1,169 ± 294	1,043 ± 525
8	4.55 ± 0.80	8.35 ± 2.36	142 ± 30	176 ± 78
24	0	0.33 ± 0.57	3.62 ± 1.63	3.67 ± 3.63

n = 3

Table 2. Pharmacokinetic Parameters for Lidocaine for Female Rats on Day 17 of Gestation

Parameter	Dose			
	15 mg/kg	30 mg/kg	60 mg/kg	75 mg/kg
C_{max} (ng/mL)	1,267	2,060	2,788	3,019
T_{max} (hr)	0.5	0.5	1	0.5
AUC_{0-24} (ng*hr/mL)	3,281	5,595	11,408	11,847
r^2	0.9750	0.8786	0.9725	0.9818
k_e (hr^{-1})	0.8435	0.3521	0.2846	0.2859
$t_{1/2}$ (hr)	0.8	2.0	2.4	2.4

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Macroscopic observations at terminal necropsy mirrored the clinical observations noted in the table above, i.e., limited to scabbing of the skin and sparse hair at the high dose lidocaine and tetracaine groups.

Two of 5 animals in the tetracaine 5 mg/kg/day dams were not pregnant; however, five of five dams in the 10 mg/kg tetracaine group were pregnant. One female in the 75 mg/kg lidocaine group was not pregnant, while one animal died while pregnant.

There were no treatment related abortions, early deliveries, complete resorptions or differences in the number of females with viable fetuses at day 20 of gestation. There were two dams in the vehicle group that had only 2 or 3 implantation sites and one dam in the 15 mg/kg lidocaine group with 2 implantation sites. These values likely contributed to some of the variability within these groups.

There were no significant differences in the mean percent post-implantation loss nonviable fetuses, resorptions (early, late or combined) between groups.

Mean gravid uterine weights in dams treated with 60 and 75 mg/kg lidocaine were increased 80% and 66% over controls (the former being statistically significant). In

contrast, there were no significant changes in mean final body weights, adjusted final body weights, weight change from Day 0 or adjusted weight change from day 0 between groups. There were no treatment-related changes in fetal weight (males, females or combined) between treatment groups.

The maternal and developmental observations at the time of uterine examination and fetal observations are summarized in the table below:

Summary of Maternal and Developmental Observations at Uterine Examination (# times observed/total number of animals affected)

Group	Veh	Lidocaine				Tetracaine			
N	4	4	5	5	3	5	5	3	5
Dose (mg/kg)	0	15	30	60	75	1	2	5	10
Pregnancy index (%)	80	80	100	100	80	100	100	60	100
Corpora Leutea	10.5	14.8	13.6	15.2	19.3*	14.0	15.8	17.7	15.2
Implantation Sites	7.3	9.8	13.2*	13.6*	13.0	13.0*	13.6*	14.0*	13.4*
Preimplantation Loss	37.3	36.9	2.6	9.5	30.4	6.8	12.5	18.8	11.6
Viable Fetuses	6.8	9.5	12.8*	13.2*	12.7	12.2	13.2*	13.3*	13.0*
Fetal Sex Ratio	51.1	24.8	59.0	49.1	41.2	51.8	53.2	47.3	46.5
% Post implantation loss	14.6	2.1	3.0	3.1	2.8	6.7	2.9	4.8	3.1
Litter Size	6.8	9.5	12.8	13.2*	12.7*	12.2	13.2*	13.3*	13.0*
Gravid Uterine Wt (g)	42.8	57.5	73.2	77.4*	71.3	72.4	80.4*	81.7*	73.4*
No. Litters Evaluated	4	4	5	5	3	5	5	3	5
No. Fetuses Evaluated	27	38	64	66	38	61	66	40	65

Offspring (malformations, variations, etc.): The total number of litters and fetuses evaluated for external malformations and developmental variations are recorded in the table above. There were no external signs of malformations or variations observed at any dose of lidocaine or tetracaine.

Study title: Pilot Prenatal Developmental Toxicity Study in New Zealand White Rabbits (With Toxicokinetics)

Key study findings: Female New Zealand Rabbits were treated with either lidocaine or tetracaine on Gestation Day 7 through 20 and the maternal and fetal effects were examined with the following key findings:

1. Maternal toxicity to a varying degree was detected following both lidocaine and tetracaine at all doses of both test articles.
2. Developmental toxicity was not noted at any dose of either test article.
3. There was no effect of either lidocaine or tetracaine on the pregnancy rate, delivery time, or maternal macroscopic pathology.
4. There was no evidence for teratogenicity following either lidocaine or tetracaine at doses up to 60 mg/kg and 10 mg/kg, respectively.
5. Based upon these findings, dose levels of 1, 5 and 15 mg/kg lidocaine and 1 and 5 mg/kg/day of tetracaine were chosen for the definitive Segment II study in rabbits.

Study no.: 925-013
Volume #, and page #: Volume 4, Page 1
Conducting laboratory and location: [redacted]
Date of study initiation: January 8, 2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Lidocaine base, Lot 811D0013, Tetracaine base, Batch 721724, [redacted]

Methods

Doses: Doses of lidocaine of 30, 60, 90 and 120 mg/kg/day were initially attempted, however, the doses were reduced to 15 and 75 mg/kg, respectively due to mortality. Tetracaine doses were 1, 2, 5 or 10 mg/kg.

Species/strain: White New Zealand Rabbits, female [Hra: (NZW) SPF]

Number/sex/group: 6 per group

Route, formulation, volume, and infusion rate: Subcutaneous, phosphate buffer, volume of 1 ml/kg.

Satellite groups used for toxicokinetics: None

Study design: Nine groups of six time-mated female rabbits were administered test article on gestation days 7 to 20 via a subcutaneous injection to the scapular or lumbar regions of the back (see table below).

Group	Test Article	Dose (mg/kg/day)	Number of Animals	Mortality
1	Control	0	6	0
2	Lidocaine	30	6	0
3	Lidocaine	60	6	3
4	Lidocaine	90 – intended dose, but due to mortality lowered to 15	1 at 90 mg/kg 5 at 15 mg/kg	1 at 90 1 at 15
5	Lidocaine	120 – intended dose, but due to mortality lowered to 75	2 at 120 mg/kg 4 at 75 mg/kg	2 at 120 4 at 75
6	Tetracaine	1	6	0
7	Tetracaine	2	6	0
8	Tetracaine	5	6	0
9	Tetracaine	10	6	0

Parameters and endpoints evaluated:

Mortality and Cage Side Observations: Animals were observed twice daily for mortality and clinical signs, a detailed clinical examination was given daily from Days 7 through 29 of gestation.

Body Weight and Body Weight Changes: Body weights were recorded on Days 0, 7, 10, 13, 16, 18, 21, 25 and 29 of gestation. Individual body weight changes were calculated for the following intervals: 0-7, 7-10, 10-13, 13-16, 16-18, 18-21, 21-25, 25-29, 7-21, 21-25, 25-29, 21-29 and 0-29. Adjusted body weight (Day 29 body weight – gravid uterine weight) and adjusted body weight change (Days 0-29 of gestation) were also calculated.

Food Consumption: Food consumption was recorded daily and reported on the corresponding body weight intervals.

Toxicokinetics: Blood samples were collected on Day 20 of gestation prior to exposure and at 0.5, 1, 2 and 4 hours after treatment. Plasma samples were collected at each of the scheduled times from three animals in each dose group, except a pre-dose sample from a fourth animal. There were no animals surviving in Group 5 (75 or 120 mg/kg/day) on Day 20 of gestation and therefore no toxicokinetic data either. Blood samples were collected into vacutainers with potassium EDTA as anticoagulant and neostigmine, an esterase inhibitor (to prevent the hydrolysis of tetracaine). Levels of lidocaine and tetracaine were determined via liquid chromatography, double mass spectrometry method (LC/MS/MS). Limits of detection were 10 ng/ml and 0.9 ng/ml for lidocaine and tetracaine, respectively.

Postmortem Study Evaluations:

Maternal Necropsy: Complete necropsy was performed on all does. Gross lesions were saved and the carcass was discarded.

Ovarian and Uterine Examinations: On Day 29, each female was euthanized by sodium pentobarbital injection and exsanguination and immediately subjected to cesarean section. The skin was reflected from the ventral midline incision to examine mammary tissue and locate any subcutaneous masses. The abdominal cavity was then opened and the uterus exposed. Location of viable and non-viable fetuses, early and late resorptions, position of the cervix and total implantations were recorded. The number of corpora lutea on each ovary was recorded. The fetuses were removed and the placenta was grossly examined.

Fetal Examinations: Fetuses were individually weighed and examined for external malformations and variations. Fetuses with external malformations and or developmental variations were preserved for possible further examination. All other fetuses were euthanized and discarded.

Results

Mortality (dams): The summary of mortalities following lidocaine treatment is presented in the table below:

Incidence of Deaths of Rabbits following Lidocaine Treatment							
Dose	0	15	30	60	75	90	120
Deaths	0/6	1/5	0/6	4/6	4/4	1/1	2/2

All rabbits treated with tetracaine survived to their scheduled termination.

Clinical signs (dams): A dose of 120 mg/kg day resulted in rapid breathing and marked decreases in activity of the dams to the point of prostration with clonic convulsions in two animals. The animals dosed at 60, 75 and 90 mg/kg exhibited these same signs. Following a dose of 30 mg/kg lidocaine there was decreased activity noted in four animals on a single day with rapid breathing in 3 of 4 animals and 2 animals on most

dosing days. One animal treated with 15 mg/kg lidocaine demonstrated decreased activity, prostration, clonic convulsions and rapid breathing.

Body weight (dams): There were test article-related changes in body weight and body weight change at all doses of lidocaine and tetracaine. Body weight gains in the 15, 30 and 60 mg/kg lidocaine group over Days 7 to 21 of gestation were 155, 138 and 43 grams, respectively. This is compared to 270 grams weight in the control animals. Body weight gains in the 1, 2, 5 and 10 mg/kg tetracaine over Days 7 through 21 of gestation were 207, 215, 205 and -108 grams respectively compared to 270 grams weight in the controls.

Food consumption (dams): Maternal food consumption during gestation was decreased compared to controls at all dose levels of lidocaine during days 7 to 21 of gestation. Food consumption was decreased significantly at the 10 mg/kg/day tetracaine. Food consumption during the pretreatment and posttreatment periods was considered similar for all groups.

Toxicokinetics:

Figure 1. Mean Lidocaine Concentrations for Female Rabbits on Day 20 of Gestation (linear plot)

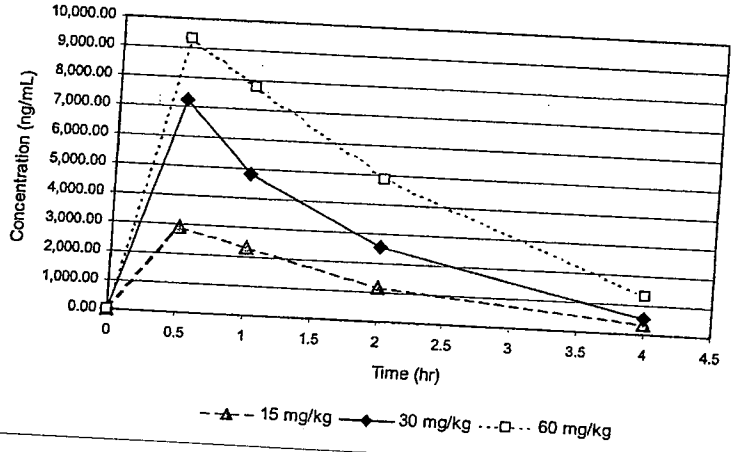


Table 1. Mean Plasma Concentrations of Lidocaine in Female Rats on Day 20 of Gestation

	Lidocaine Concentration (ng/mL)		
Time (hr)	15 mg/kg Mean ± SD	30 mg/kg Mean ± SD	60 mg/kg Mean ± SD
0	3.33 ± 6.67*	0	7.35 ± 12.72
0.5	2,928 ± 348	7,209 ± 1,922	9,327 ± 5,686
1	2,306 ± 82	4,821 ± 736	7,752 ± 2,908
2	1,164 ± 131	2,550 ± 338	4,843 ± 1,954
4	378 ± 167	556 ± 217	1,332 ± 458

n = 3, except as noted
* n = 4

Figure 3. Mean Tetracaine Concentrations for Female Rabbits on Day 20 of Gestation (linear plot)

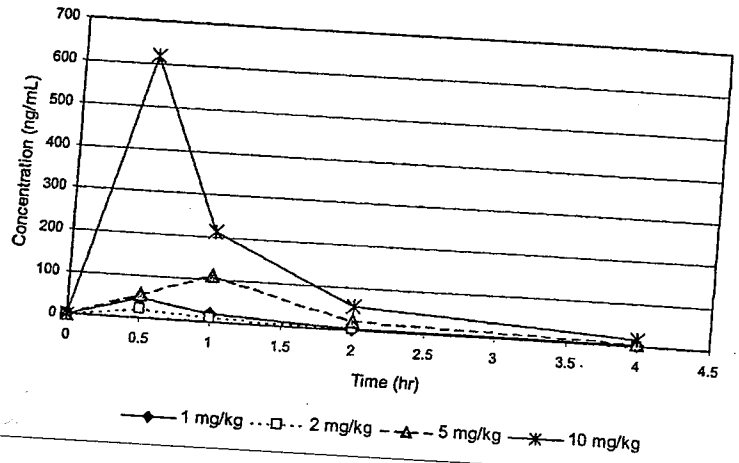


Table 2. Mean Plasma Concentrations of Tetracaine in Female Rabbits on Day 20 of Gestation

Time (hr)	Tetracaine Concentration (ng/mL)			
	1 mg/kg Mean ± SD	2 mg/kg Mean ± SD	5 mg/kg Mean ± SD	10 mg/kg Mean ± SD
0	0	0	0	0
0.5	45.68 ± 35.91	22.78 ± 20.61	56.95 ± 29.70	618.30 ± 151.48
1	19.86 ± 22.74	9.68 ± 8.56	110.81 ± 108.50	212.86 ± 110.03
2	3.40 ± 0.95	3.91 ± 3.40	22.54 ± 14.45	57.07 ± 27.83
4	1.94 ± 2.18	0.70 ± 0.61	7.67 ± 2.01	16.11 ± 11.29

n = 3 in all cases

Table 3. Mean Pharmacokinetic Parameters for Lidocaine for Female Rabbits on Day 20 of Gestation

Lidocaine Parameter	15 mg/kg Dose Mean ± SD	30 mg/kg Dose Mean ± SD	60 mg/kg Dose Mean ± SD
C_{max} (ng/mL)	2,928 ± 348	7,209 ± 1,922	10,703 ± 4,624
T_{max} (hr)	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.3
AUC_{0-24} (ng*hr/mL)	8,001 ± 1,007	16,477 ± 1,341	28,954 ± 9,675
k_e (hr^{-1})	0.5322 ± 0.2893	0.7454 ± 0.2015	0.5384 ± 0.3029
$t_{1/2}$ (hr)	1.8 ± 1.3	1.0 ± 0.3	1.7 ± 1.2

n = 3 in all cases

Table 4. Mean Pharmacokinetic Parameters for Tetracaine for Female Rabbits on Day 20 of Gestation

Parameter	1 mg/kg Dose Mean ± SD	2 mg/kg Dose Mean ± SD	5 mg/kg Dose Mean ± SD	10 mg/kg Dose Mean ± SD
C_{max} (ng/mL)	45.7 ± 35.9	22.8 ± 20.6	123.2 ± 99.3	618.3 ± 151.5
T_{max} (hr)	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.3	0.5 ± 0.0
AUC_{0-24} (ng*hr/mL)	59.2 ± 46.0	30.5 ± 26.5	209.0 ± 56.3	683.5 ± 216.2
k_e (hr^{-1})	0.6452 *	0.8697 *	0.6488 *	0.8625 ± 0.0887
$t_{1/2}$ (hr)	1.1 *	0.8 *	1.2 *	0.8 ± 0.1

n = 3, except as noted. SD not calculated for n < 3.
* n = 2

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Maternal toxicity: The number of corpora lutea, implantation sites, preimplantation loss, viable fetuses, litter size, and resorptions were similar between the control group and the groups treated with lidocaine or tetracaine. There were no viable fetuses at 75 mg/kg/day lidocaine. An **increase** in post-implantation loss was seen at dose levels of 30 and 60 mg/kg/day lidocaine and 1 mg/kg/day tetracaine. Gravid uterine weights, adjusted Day 29 gestation body weights, and adjusted body weight changes from Day 0 were comparable to controls except for the 60 mg/kg/day for the treated lidocaine and tetracaine groups were

Summary of Maternal and Developmental Observations at Uterine Examination (# times observed/total number of animals affected)

Group	**Veh**	**Lidocaine**				**Tetracaine**			
N	**6**	**6**	**6**	**6**	**6**	**5**	**5**	**3**	**5**
Dose (mg/kg)	**0**	**15**	**30**	**60**	**75**	**1**	**2**	**5**	**10**
Pregnancy index (%)	100	100	100	100	100	100	100	100	100
No. Died Pregnant	0	2	0	3	6	0	0	0	0
No. Abortions	0	0	0	1	0	0	0	0	1
% Post implantation loss	3.60	2.78	14.58	15.66	NA	16.01	7.20	7.08	0
Litter Size	9.3	7.8	7.0	9.0	NA	7.3	8.8	8.7	8.4

No. Litters Evaluated	6	4	6	2	NA	6	6	6	5
No. Fetuses Evaluated	56	31	42	17	NA	44	53	52	42
Mean Fetal Body Wt	41.95	44.83	39.73	36.02	NA	42.73	40.93	43.15	45.56
Total Malformations									
No. Litters (%)	0	0	0	0	NA	0	0	0	0
No. Fetuses (%)	0	0	0	0	NA	0	0	0	0
Total Variations									
No. Litters (%)	0	0	1	0	NA	0	0	0	0
No. Fetuses (%)	0	0	1	0	NA	0	0	0	0

NA = not applicable or not available (all animals died prior to scheduled euthanasia.

Offspring (malformations, variations, etc.):

Fetal body weights: Fetal body weights for the lidocaine and tetracaine groups did not differ statistically from controls. Mean fetal weights at 60 mg/kg/day of lidocaine was lower than controls and thought to be treatment related. Following dosing of 15 mg/kg/day of lidocaine and 10 mg/kg/day of tetracaine, fetal body weight was slightly higher than controls.

External examinations: The only external malformation noted in this study was an abnormal flexure in the forelimb and hind limb of a single fetus from 30 mg/kg/day lidocaine group. This abnormality was not considered test-article related.

Study title: Study for Effects on Embryo-Fetal Development in Rats

Key study findings: The effect of subcutaneous lidocaine (5, 15 and 60 mg/kg/day), tetracaine (5 and 10 mg/kg/day) and the eutectic combination of the two (10 mg/kg/day each) on the embryofetal development of the rat were examined with the following key findings:

1. Maternal toxicity was noted at the high doses of lidocaine, tetracaine and the combination, indicating that the study is a valid assessment of the teratogenic potential of these drugs.
2. There was no evidence of teratogenicity in any treatment under the conditions of this assay.
3. The NOAEL for maternal toxicity was 5 mg/kg/day of tetracaine and 15 mg/kg/day of lidocaine. These doses correspond to 30 and 90 mg/m^2, on a body surface area basis.
4. The NOAEL for developmental effects was 10 mg/kg/day tetracaine, 60 mg/kg/day lidocaine and 10 mg/kg/day each in a eutectic mixture. These doses correspond to 60, 360 and 60/60 mg/m^2, respectively, on a body surface area basis.

Study no.: 925-015
Volume #, and page #: Volume 5, Page 1
Conducting laboratory and location: [redacted]

Date of study initiation: February 26, 2003
GLP compliance: Yes

QA reports: yes (X) no ( )
Drug, lot #, and % purity: Tetracaine base, Batch # 721724, [redacted]
Lidocaine base, Lot # 811D0013, [redacted]

Methods

Doses: Lidocaine 5, 10 and 60 mg/kg, s.c.
Tetracaine 5 and 10 mg/kg, s.c.
Lidocaine/Tetracaine 10/10 mg/kg, s.c.

Species/strain: Sprague-Dawley rats [[redacted]CD (SD) IGS BR]

Number/sex/group: 25 as outlined in the table below:

Group Assignment

Group Number	Dose Level (mg/kg/day)	Number of Time-mated Female Rats
1	0 (Vehicle Control)	25
2	5 (Lidocaine)	25
3	10 (Lidocaine)	25
4	60 (Lidocaine)	25
5	5 (Tetracaine)	25
6	10 (Tetracaine)	25
7	10/10 (Lidocaine/Tetracaine)	25

Route, formulation, volume, and infusion rate: Subcutaneous, vehicle was phosphate buffered saline, pH = 6.0 to 6.2, volume of 1 ml/kg. The dosing formulations were determined to be stable for 14 days when refrigerated via preliminary studies.

Satellite groups used for toxicokinetics: Not completed.

Study design: Test article and vehicle control administration began on Day 6 of gestation and continued through to include Day 17 of gestation. Individual doses were based on the most recent body weight. Test article/vehicle was administered subcutaneously in the scapular and lumbar regions of the lower back via a 26-gauge hypodermic needle. Dosing was alternated from left to right.

Parameters and endpoints evaluated:

Mortality and Clinical Signs: Animals were observed twice daily for morbidity, mortality, signs of injury and availability of food and water. Detailed clinical examinations were conducted daily from Days 6 through 20 of gestation.

Body Weights: Body weights were recorded on Days 0, 6, 9, 12, 15, 18 and 20 of gestation. Body weight changes were calculated for the following gestation day intervals: 0-6, 6-9, 9-12, 12-15, 15-18, 18-20, 6-20 and 0-20. Adjusted body weight (Day 20 gestation body weight minus gravid uterine weight) and adjusted body weight change (Days 0-20 of gestation) were also calculated.

Food Consumption: Food consumption was recorded on the corresponding body weight days and calculated for the same intervals as body weight change.

Post Mortem Evaluations: On Day 20, Dams were sacrificed by carbon dioxide inhalation and immediately subjected to cesarean section. Maternal necropsy, ovarian and uterine examinations were completed. The following were recorded: gravid

uterine weight, location of viable and nonviable fetuses, early and late resorptions, position of cervix, total number of implantations, number or corpora lutea on each ovary.

Teratogenic Examinations: Fetuses were individually weighed, sexed, tagged and examined for external malformations and variations. Fetuses were euthanized via intraperitoneal injection of sodium barbital. Approximately half were placed in Bouin's solution, the remaining in alcohol. Skeletal malformations and developmental variations were noted and classified as such under the supervision of a developmental toxicologist.

Statistical analysis: Statistical analysis was conducted according to the following table:

Statistical Analysis Methods	
Endpoint	**Analysis**
Parental In-life Data	
Gestation Body Weights	Group Pair-wise Comparisons
Gestation Body Weight Changes	Group Pair-wise Comparisons
Gestation Food Consumption	Group Pair-wise Comparisons
Adjusted Body Weights	Group Pair-wise Comparisons
Adjusted Body Weight Changes (Days 0-20)	Group Pair-wise Comparisons
Fertility Indices	
Pregnancy Index	Fisher's Exact Test
Uterine and Ovarian Exam	
Gravid Uterine Weights	Group Pair-wise Comparisons
Corpora Lutea/dam	Group Pair-wise Comparisons
Total Implantations/dam	Group Pair-wise Comparisons
Fetal Sex Ratio (% males/litter)	Arcsin-Square-Root Transformation
Litter Size/dam	Group Pair-wise Comparisons
Viable Fetuses/dam	Group Pair-wise Comparisons
Nonviable Fetuses /dam	Descriptive Statistics
Total Number Resorptions/dam	Group Pair-wise Comparisons
Number Early Resorptions/dam	Group Pair-wise Comparisons
Number Late Resorptions/dam	Group Pair-wise Comparisons
% Preimplantation Loss (mean/dam)	Arcsin-Square-Root Transformation
% Postimplantation Loss (mean/dam)	Arcsin-Square-Root Transformation
Mean Fetal Body Weights	Covariate Analysis
Malformations by finding and exam type (external, visceral, and skeletal) – litter incidence[a]	Fisher's Exact Test
Variations by finding and exam type (external, visceral, and skeletal) – litter incidence[a]	Fisher's Exact Test
Total Malformations (external, visceral, and skeletal combined) – litter incidence[a]	Fisher's Exact Test

[a]Fetal and litter incidences are reported, but only the litter incidences were statistically analyzed.

Results

Mortality (dams): Five pregnant rats treated with 10 mg/kg tetracaine were found dead on Days 6, 8, 10, 10 and 12 of gestation. These deaths were considered to be test article related.

Clinical signs (dams): Lidocaine treatment-related clinical findings were limited to prostration in one treated dam at the high dose on one occasion. In addition, dams in the high dose lidocaine group were frequently described as having hair loss or sparse hair and scabbed areas of the skin on the dorsal surface. Behavioral observations in the tetracaine treatment group included decreased activity and convulsions following 10 mg/kg doses.

Summary of Clinical Observations in the Dams (# times observed/total number of animals affected)

Group	Vehicle	Lidocaine			Tetracaine		Lido/Tet
N	25	25	25	25	25	24	25
Dose (mg/kg)	0	5	15	60	5	10	10/10
Behavior							
Activity Decreased	0/0	0/0	0/0	0/0	0/0	158/23	195/25
Behavior aggressive	0/0	0/0	0/0	0/0	0/0	1/1	1/1
Convulsions – clonic	0/0	0/0	0/0	0/0	0/0	3/3	1/1
Licking excessive	0/0	0/0	0/0	0/0	0/0	1/1	0/0
Prostration	0/0	0/0	0/0	1/1	0/0	125/23	186/25
Salivation	0/0	0/0	0/0	0/0	0/0	2/2	0/0
Skin*							
Hair sparse, lumbar	0/0	0/0	1/1	49/9	0/0	0/0	3/1
Hair absent, dorsal	0/0	0/0	0/0	36/4	0/0	0/0	0/0
Scabbed area, dorsal	0/0	0/0	0/0	127/15	12/2	39/7	137/13
Respiration							
Rapid breathing	0/0	0/0	0/0	0/0	0/0	121/23	156/25

* Only selected body regions are described here. The study report breaks down a large number of regions which were not deemed necessary to reproduce here.

Body weight (dams): Occasional decreases in body weight gain were observed following treatment with 10 mg/kg of the eutectic mixture of lidocaine/tetracaine during some of the internals. There were no test article related body weight or body weight gains noted at any dose levels in any group.

Food consumption (dams): There were no test-article related changes in maternal food consumption during gestation.

Toxicokinetics: Not completed in this study (see pilot study data).

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Post-mortem necroscopic observations included scabbing and red discoloration at some of the injection sites in the 60 mg/kg/day lidocaine, 10 mg/kg/day tetracaine and the 10 mg/kg/day eutectic mixture of lidocaine and tetracaine. These findings were likely attributed to the test article.

There were no significant differences between groups in gravid uterine weights, adjusted Day 20 gestation body weights and body weight gains over Days 0-20.

There were no abortions or early deliveries noted, and pregnancy rate was similar across all groups (96-100%) except for the 5 mg/kg/day lidocaine group and the 5 mg/kg/day tetracaine group (84% and 88%, respectively). One female in the 60 mg/kg/day lidocaine

group and one female in the 5 mg/kg/day lidocaine group had all resorptions, while all groups had 20 or more pregnant animals with viable fetuses.

Summary of Maternal and Developmental Observations at Uterine Examination							
Group	**Vehicle**	**Lidocaine**			**Tetracaine**		**Lido/Tet**
Dose (mg/kg)	**0**	**5**	**15**	**60**	**5**	**10**	**10/10**
Endpoint							
# Females on study	25	25	25	25	25	25	25
# not pregnant	0	4	0	1	3	0	1
# pregnant	25	21	25	24	22	25	24
Pregnancy Index (%)	100	84	100	96	88	100	96
# Died Pregnant	0	0	0	0	0	5	0
# Abortions	0	0	0	0	0	0	0
# Early deliveries	0	0	0	0	0	0	0
# Females with all resorptions	0	1	0	1	0	0	0
# females with viable fetuses Day 20 gestation	25	21	25	24	22	20	24
Mean Corpora Lutea	13.1	13.6	13.7	14.2	13.6	13.0	15.3*
Mean Implantation sites	12.3	12.3	12.7	12.7	12.5	12.3	12.3
Mean Preimplantation loss %	7.86	5.73	6.77	6.24	6.17	5.04	17.41
Mean Viable fetuses	11.6	11.8	12.2	12.0	12.0	11.6	11.9
Mean Fetal sex ratio (% males)	54.3	46.0	48.1	45.0	53.7	52.2	50.0
Mean postimplantation loss (%)	5.08	8.35	3.96	8.87	4.66	5.91	2.28
Mean Nonviable fetuses	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean Litter size	11.6	11.8	12.2	12.0	12.0	11.6	11.9
Mean Resorptions (early + late)	0.7	0.5	0.5	0.7	0.6	0.8	0.3
Mean Resorptions (early)	0.7	0.5	0.5	0.7	0.6	0.8	0.3
Mean Resorptions (late)	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* $p < 0.05$ compare to control

Offspring (malformations, variations, etc.): There were no significant differences in the mean fetal weights (males, females or combined) between treatment groups. Further, there were no differences in the incidence of external malformations or variations that could be attributed to drug treatment. There was a slight increase in the number of fetuses with unossified hyloid of the skull in the lidocaine/tetracaine eutectic mixture group, however, this was not statistically significant and within historical control range (maximum of 6.9% of fetuses and 24% litters affected).

Summary of Fetal External Observations (Incidence expressed as the number of fetuses affected)							
Group	**Vehicle**	**Lidocaine**			**Tetracaine**		**Lido/Tet**
Dose (mg/kg)	**0**	**5**	**15**	**60**	**5**	**10**	**10/10**
# of Litters Evaluated	25	20	25	23	22	20	24
# of Fetuses Evaluated	290	248	304	288	263	231	286
Body							
Entire, thoracogastroschisis	0	0	0	0	1	0	0
Forelimbs							
Digits, ectrodactyly	0	0	0	0	1	0	0
Hindlimbs							
Entire, abnormal flexure	0	0	0	0	1	0	0
Hind paw, edema	0	0	0	0	0	1	0
Tail							

Entire, absent	0	0	0	0	0	0	1
Summary of External Obs.							
Total Malformations							
# Litters (%)	0	0	0	0	1	0	1
# Fetuses (%)	0	0	0	0	1	0	1
Total Variations							
# Litters (%)	0	0	0	0	1	0	0
# Fetuses (%)	0	0	0	0	1	0	0

Summary of Fetal Visceral Observations
(Incidence expressed as the number of fetuses affected)

Group	Vehicle	Lidocaine	Tetracaine	Lido/Tet
Dose (mg/kg)	**0**	**60**	**10**	**10/10**
# of Litters Evaluated	24	23	20	24
# of Fetuses Evaluated	143	147	116	143
Kidney				
Increased renal pelvic cavitation	0	3	0	1
Ureter, dilated	0	1	0	0
Summary of Visceral Obs.				
Total Malformations				
# Litters (%)	0	0	0	0
# Fetuses (%)	0	0	0	0
Total Variations				
# Litters (%)	0	2	0	1
# Fetuses (%)	0	3	0	1

Summary of Fetal Skeletal Observations
(Incidence expressed as the number of fetuses affected)

Group	Vehicle	Lidocaine	Tetracaine	Lido/Tet
Dose (mg/kg)	**0**	**60**	**10**	**10/10**
# of Litters Evaluated	25	23	20	24
# of Fetuses Evaluated	147	141	115	143
Pelvic Girdle				
Ischium, incompletely ossified	0	1	1	0
Pubic, not ossified	1	0	0	0
Rib(s)				
Rib(s), bent	0	1	0	0
Rib(s), rudimentary	15	11	13	14
Rib(s), unilateral full rib	3	0	0	0
Skull				
Hyoid, not ossified	1	1	1	**5**
Sternum				
Sternebra(e), misaligned	1	2	1	1
Sternebra(e), not ossified	15	13	14	7
Summary of Skeletal Obs.				
Total Malformations				
# Litters (%)	0	0	0	0
# Fetuses (%)	0	0	0	0
Total Variations				
# Litters (%)	12	17	13	15
# Fetuses (%)	32	25	27	25

Study title: Study for Effects on Embryo-Fetal Development in New Zealand White Rabbits

Key study findings: The effect of subcutaneous lidocaine (1, 5 and 15 mg/kg/day), tetracaine (1 and 5 mg/kg/day) and the eutectic combination of the two (5 mg/kg/day each) on the embryofetal development of the rabbit were examined with the following key findings:

a. Maternal toxicity was noted at the high doses of lidocaine, tetracaine and the combination, indicating that the study is a valid assessment of the teratogenic potential of these drugs.
b. There was no evidence of teratogenicity in any treatment under the conditions of this assay.
c. The NOAEL for maternal toxicity was 1 mg/kg/day of tetracaine and 15 mg/kg/day of lidocaine. These doses correspond to 12 and 180 mg/m^2, on a body surface area basis.
d. The NOAEL for developmental effects was 5 mg/kg/day tetracaine, 15 mg/kg/day lidocaine and 5 mg/kg/day each in a eutectic mixture (highest dose tested). These doses correspond to 60, 180 and 60/60 mg/m^2, respectively, on a body surface area basis.
e.

Study no.: 925-016
Volume #, and page #: Volume 6, Page 1
Conducting laboratory and location: [redacted]

Date of study initiation: February 26, 2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Tetracaine base, Batch # 721724, [redacted]
Lidocaine base, Lot # 811D0013,

Methods

Doses: Lidocaine 1, 5 and 15 mg/kg, s.c.
Tetracaine 1 and 5 mg/kg, s.c.
Lidocaine/Tetracaine 5/5 mg/kg, s.c.

Species/strain: New Zealand White Hra(NZW)SPF Rabbits [redacted]

Number/sex/group: 23/group as outlined in the table below:

Group Assignment		
Group Number	Dose Level (mg/kg/day)	Number of Time-mated Female Rabbits
1	0 (Vehicle Control)	23
2	1 (Lidocaine)	23
3	5 (Lidocaine)	23
4	15 (Lidocaine)	23
5	1 (Tetracaine)	23
6	5 (Tetracaine)	23
7	5/5 (Lidocaine/Tetracaine)	23

Route, formulation, volume, and infusion rate: Subcutaneous, vehicle was phosphate buffered saline, pH = 6.0 ± 0.2, volume of 1 ml/kg. The dosing formulations were determined to be stable for 14 days when refrigerated via preliminary studies.

Satellite groups used for toxicokinetics: Not completed in this study.

Study design: Test article and vehicle control administration began on Day 7 of gestation and continued through to include Day 20 of gestation. Individual doses were based on the most recent body weight. Test article/vehicle was administered via a 26-gauge hypodermic needle subcutaneously in the scapular and lumbar regions of the lower back. Dosing was alternate form left to right.

Parameters and endpoints evaluated:

Mortality and Clinical Signs: Animals were observed twice daily for morbidity, mortality, signs of injury and availability of food and water. Detailed clinical examinations were conducted daily from Days 7 through 29 of gestation.

Body Weights: Body weights were recorded on Days 0, 7, 10, 13, 16, 18, 21, 25 and 29 of gestation. Body weight changes were calculated for the following gestation day intervals: 0-7, 7-10, 10-13, 13-16, 16-18, 18-21, 21-29 and 0-29. Adjusted body weight (Day 29 gestation body weight minus gravid uterine weight) and adjusted body weight change (Days 0-29 of gestation) were also calculated.

Food Consumption: Food consumption was recorded daily and reported on the corresponding body weight days and calculated for the same intervals as body weight intervals.

Post Mortem Evaluations: On Day 29, dams were sacrificed by sodium pentobarbital injection followed by exsanguinations from the femoral blood vessels and immediately subjected to cesarean section. Maternal necropsy, ovarian and uterine examinations were completed. The following were recorded: gravid uterine weight, location of viable and nonviable fetuses, early and late resorptions, position of cervix, total number of implantations, number or corpora lutea on each ovary.

Teratogenic Examinations: Fetuses were individually weighed, sexed, tagged and examined for external malformations and variations. Fetuses were euthanized via intraperitoneal injection of sodium barbital. Approximately half were placed in Bouin's solution, the remaining in alcohol. Skeletal malformations and developmental variations were noted and classified as such under the supervision of a developmental toxicologist.

Statistical analysis: Statistical analysis was conducted according to the following table:

Statistical Analysis Methods	
Endpoint	**Analysis**
Parental In-life Data	
Gestation Body Weights	Group Pair-wise Comparisons
Gestation Body Weight Changes	Group Pair-wise Comparisons
Gestation Food Consumption	Group Pair-wise Comparisons
Adjusted Body Weights	Group Pair-wise Comparisons
Adjusted Body Weight Changes (Days 0-29)	Group Pair-wise Comparisons
Fertility Indices	
Pregnancy Index	Fisher's Exact Test
Uterine and Ovarian Exam	
Gravid Uterine Weights	Group Pair-wise Comparisons
Corpora Lutea/doe	Group Pair-wise Comparisons
Total Implantations/doe	Group Pair-wise Comparisons
Fetal Sex Ratio (% males/litter)	Fisher's Exact Test
Litter Size/doe	Group Pair-wise Comparisons
Viable Fetuses/doe	Group Pair-wise Comparisons
Nonviable Fetuses /doe	Descriptive Statistics
Total Number Resorptions/doe	Group Pair-wise Comparisons
Number Early Resorptions/doe	Group Pair-wise Comparisons
Number Late Resorptions/doe	Group Pair-wise Comparisons
% Preimplantation Loss (mean/doe)	Arcsin-Square-Root Transformation
% Postimplantation Loss (mean/doe)	Arcsin-Square-Root Transformation
Mean Fetal Body Weights	Covariate Analysis
Malformations by finding and exam type (external, visceral, and skeletal) – litter incidence[a]	Fisher's Exact Test
Variations by finding and exam type (external, visceral, and skeletal) – litter incidence[a]	Fisher's Exact Test
Total Malformations (external, visceral, and skeletal combined) – litter incidence[a]	Fisher's Exact Test
[a]Fetal and litter incidences are reported, but only the litter incidences were statistically analyzed.	

Results

Mortality (dams): One pregnant animal in the 5 mg/kg/day lidocaine group was sacrificed in extremis on Day 15 due to a hind limb impairment. This was not thought to be treatment-related. One pregnant animal in the 15 mg/kg/day lidocaine group was found dead on Day 21 and one pregnant animal in the 5 mg/kg/day tetracaine group was found dead on Day 17. The tetracaine animal had decreased activity, absence of feces and inappetance prior to death. There were no clinical signs in the 15 mg/kg/day lidocaine animal prior to death. The death of the animal in the tetracaine group was considered to be test-article related. However, the sponsor does not consider the death of the animals treated with lidocaine to be treatment-related. As dosing was completed on study day 20, this conclusion appears to be reasonable.

Clinical signs (dams): Lidocaine treatment-related clinical findings were limited to aggressive behavior in 2 dams treated with the high dose, hair absent/sparse in various regions and scabbed areas on the back that where likely related to injection sites

irritation. Behavioral changes in the tetracaine treated animals included decreased activity, ataxia, aggressive behavior, convulsions and prostration. The tetracaine-induced prostration, ataxia and to a lesser extent convulsions appear to have been increased by the addition of the lidocaine.

Summary of Clinical Observations in the Dams (# times observed/total number of animals affected)

Group	Vehicle	Lidocaine			Tetracaine		Lido/Tet
N	23	23	23	23	23	23	23
Dose (mg/kg)	0	1	5	15	1	5	5/5
Behavior							
Activity Decreased	4/3	6/1	3/2	11/2	0/0	106/22	216/23
Activity Increased	0/0	0/0	0/0	0/0	0/0	0/0	1/1
Ataxia	0/0	0/0	0/0	0/0	0/0	1/1	13/8
Behavior aggressive	0/0	0/0	0/0	0/0	0/0	13/1	10/1
Convulsions – clonic	0/0	0/0	0/0	0/0	0/0	12/9	18/11
Inappetence	0/0	0/0	0/0	0/0	0/0	2/1	0/0
Prostration	0/0	0/0	0/0	0/0	0/0	87/22	163/23
Skin*							
Hair sparse, lumbar	0/0	0/0	0/0	8/1	3/1	21/3	0/0
Hair absent, lumbar	1/1	3/1	0/0	15/2	3/1	0/0	0/0
Scabbed area, lumbar	0/0	0/0	0/0	23/2	25/2	64/7	10/1
Respiration							
Rapid breathing	0/0	0/0	0/0	0/0	0/0	102/21	219/23

* Only selected body regions are described here. The study report breaks down a large number of regions which were not deemed necessary to reproduce here.

Body weight (dams): There were no test-article related changes in body weight or body weight gain with any dose of lidocaine or with the low dose of tetracaine (1 mg/kg/day). Body weight and body weight gains of the dams in the 5 mg/kg/day tetracaine group and the 5/5 mg/kg/day lidocaine/tetracaine group were lower than the controls at the Day 7-21 gestational interval for tetracaine 5 mg/kg/day and the Day 0-29 gestational interval for the high dose tetracaine and lidocaine/tetracaine groups. These changes are considered to be treatment related.

Food consumption (dams): Food consumption in the lidocaine 15 mg/kg/day dams for gestational intervals Day 7-21 and Day 0-29 were slightly lower than controls, but not statistically significant. There as a statistically significant decrease in food consumption in dams treated with 5 mg/kg tetracaine or 5/5 mg/kg/day lidocaine/tetracaine for gestational intervals Day 7-21 and Day 0-29. These changes are considered to be treatment related.

Toxicokinetics: Not completed in this study (see pilot study data).

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Post-mortem necroscopic observations included scabbing at the injection site in one 15 mg/kg/day lidocaine dam, 1 mg/kg/day tetracaine dam and 5 dams in the 5 mg/kg/day lidocaine/tetracaine group. Red discoloration at the injection sites

were also noted in one 5 mg/kg/day tetracaine dam and 2 dams in the 5/5 mg/kg/day lidocaine/tetracaine group. These findings were likely attributed to the test article.

There were no abortions or any early deliveries noted in any group, and pregnancy rate was similar across all groups (91.3-100%). The number of females with viable litters was similar across groups (ranged from 20-22). There were no statistically significant or toxicologically relevant changes in the number of corpora lutea, implantations, post-implantation loss, viable and non-viable fetuses or resorptions between groups.

Summary of Maternal and Developmental Observations at Uterine Examination

Group	Vehicle	Lidocaine			Tetracaine		Lido/Tet
Dose (mg/kg)	0	1	5	15	1	5	5/5
Endpoint							
# Females on study	23	23	23	23	23	23	23
# not pregnant	1	2	2	2	2	0	1
# pregnant	22	21	21	21	21	23	22
Pregnancy Index (%)	95.7	91.3	91.3	91.3	91.3	100	95.7
# Died Pregnant	0	0	1	1	0	1	0
# Abortions	0	0	0	0	0	0	0
# Early deliveries	0	0	0	0	0	0	0
# Females with all resorptions	0	0	0	0	0	0	0
# females with viable fetuses Day 20 gestation	22	21	20	20	21	22	22
Mean Corpora Lutea	9.6	10.4	10.2	9.8	10.5	9.7	10.9
Mean Implantation sites	9.1	9.4	9.6	9.1	9.9	9.0	10.0
Mean Preimplantation loss %	5.21	10.61	5.42	7.34	5.93	6.36	6.42
Mean Viable fetuses	8.8	9.0	9.2	8.8	9.4	8.5	9.6
Mean Fetal sex ratio (% males)	54.1	56.7	49.6	51.4	49.8	46.8	52.8
Mean postimplantation loss (%)	2.66	3.0	3.47	2.69	4.16	5.79	3.89
Mean Nonviable fetuses	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean Litter size	8.8	9.0	9.2	8.8	9.4	8.5	9.6
Mean Resorptions (early + late)	0.3	0.3	0.4	0.3	0.4	0.5	0.4
Mean Resorptions (early)	0.1	0.2	0.1	0.2	0.2	0.5	0.2
Mean Resorptions (late)	0.1	0.1	0.3	0.1	0.2	0.1	0.2

* $p < 0.05$ compare to control

Adjusted body weight and body weight gains were also not altered in dams treated with any regimen of lidocaine alone or 1 mg/kg tetracaine treatment. However, dams treated with 5 mg/kg/day tetracaine and 5/5 mg/kg/day lidocaine/tetracaine had a lower weight change from day 0 and animals in the 5/5 mg/kg/day lidocaine/tetracaine group had a lower adjusted bodyweight change from day 0 compared to control animals that was statistically significance. The magnitude of the body weight change from day 0 was approximately 28% for both the tetracaine and the tetracaine/lidocaine treatments, suggesting a primary role of tetracaine in this response.

Summary of Gravid Uterine Weight and Adjusted Body Weight Change Values							
Group	**Vehicle**	**Lidocaine**			**Tetracaine**		**Lido/Tet**
Dose (mg/kg)	**0**	**1**	**5**	**15**	**1**	**5**	**5/5**
Gravid uterine weight, kg	0.498	0.521	0.520	0.503	0.537	0.478	0.0875
Final body weight, kg	3.916	3.946	3.848	3.893	3.858	3.794	3.837
Adjusted final body weight, kg	3.418	3.425	3.328	3.389	3.321	3.318	3.323
Weight change from day 0, kg	0.538	0.540	0.515	0.519	0.477	0.390*	0.388*
Adjusted weight change from day 0, kg	0.041	0.019	-0.005	0.015	-0.060	-0.086	-0.126*

* $p < 0.05$ compared to vehicle-treated group

There were no significant differences in fetal body weight between treatment groups when examined as males, females or combined.

Offspring (malformations, variations, etc.): There were no statically significant increases in the total number of litters with **external malformations or external variations** noted under the conditions of the study. Statistical analysis of the data expressed on the basis of fetuses evaluated (rather than litters) was not completed by the sponsor. The data provided is reproduced below from the sponsor's Table 10.

Summary of Fetal External Observations (Incidence expressed as the number of fetuses affected)							
Group	**Vehicle**	**Lidocaine**			**Tetracaine**		**Lido/Tet**
Dose (mg/kg)	**0**	**1**	**5**	**15**	**1**	**5**	**5/5**
# of Litters Evaluated	22	21	20	20	21	22	22
# of Fetuses Evaluated	194	190	184	176	198	187	212
Body							
Abdomen, gastroschisis[2]	0	0	0	1	0	0	1
Entire, edema	0	0	0	0	0	0	1
Forelimbs							
Digits, ectrodactyly	0	0	0	0	0	0	1
Entire, abnormal flexure	0	0	0	0	0	0	1
Fore paw, abnormal flexure	0	1	0	0	0	0	1
Hind limb(s)							
Entire, abnormal flexure	0	0	0	0	0	0	1
Entire, malrotated	0	0	0	0	0	0	1
Tail							
Entire, absent	0	0	0	0	0	0	1
Summary of External Obs.							
Total Malformations							
# Litters (%)	0 (0)	0 (0)	0 (0)	1 (5.0)	0 (0)	0 (0)	2 (9.1)
# Fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	0 (0)	2 (0.9)
Total Variations							
# Litters (%)	0 (0)	1 (4.8)	0 (0)	0 (0)	0 (0)	0 (0)	2 (9.1)
# Fetuses (%)	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.9)

[2] Gastroschisis -A defect in the abdominal wall resulting from rupture of the amniotic membrane during physiological gut-loop herniation or, later, owing to delayed umbilical ring closure; usually accompanied by protrusion of viscera.

There were no statically significant increases in the total number of litters with **visceral malformations or visceral variations** noted under the conditions of the study. There were several rare malformations noted in a treatment group that were not seen in the concurrent controls not have they been seen in the sponsor's historical database for the facility. These include hydrocephaly (lateral ventricle), absent gallbladder, absent ureter / kidney, and smaller than normal ovary. Although rare, the sponsor noted that they occurred in only one or two animals and there were no other animals affected and therefore are not considered to be related to the test article. Statistical analysis of the data expressed on the basis of fetuses evaluated (rather than litters) was not completed by the sponsor. The absent gall bladder was also noted in one vehicle treated animal and therefore does not appear to be related to drug treatment. The absent kidney and ureter noted in the lidocaine:tetracaine group is not a common finding and was not detected in the historical control database (MARTA and MTA). However, as this finding occurs in only one animal, the evidence is rather weak that the effect could be attributed to the drug treatments.

Summary of Fetal Visceral Observations
Incidence expressed as the number of fetuses affected (%)

Group	Vehicle	Lidocaine			Tetracaine		Lido/Tet
Dose (mg/kg)	**0**	**1**	**5**	**15**	**1**	**5**	**5/5**
# of Litters Evaluated	22	21	20	20	21	22	22
# of Fetuses Evaluated	194	190	184	176	198	187	212
Gall Bladder							
Absent	1 (0.5)	2 (1.1)	0	1 (0.6)	0	0	0
Small	2 (1.0)	3 (1.6)	1 (0.5)	2 (1.1)	2 (1.0)	3 (1.6)	10 (4.7)
Kidney							
absent	0	0	0	0	0	0	1 (0.5)
Liver							
nodule	0	0	0	0	1 (0.5)	0	0
Ovary							
smaller than normal	0	0	0	0	0	1 (0.5)	0
Ureter							
Absent	0	0	0	0	0	0	1 (0.5)
Brain							
Lat ventricle, hydrocephaly	0	0	0	0	0	1 (0.5)	0
Head							
Eye, microphthalmia	0	0	0	0	0	0	1 (0.5)
Aortic arch							
Dilated	0	1 (0.5)	1 (0.5)	0	1 (0.5)	0	1 (0.5)
Lungs (both)							
Smaller than normal	0	0	0	0	0	1 (0.5)	0
Diaphragm							
Diaphramatic hernia	0	0	0	0	0	1 (0.5)	0
Intraventricular septum							
Discontinuous	0	1 (0.5)	1 (0.5)	0	1 (0.5)	0	0
Pulmonary truck							
Constricted	0	1 (0.5)	0	0	0	0	1 (0.5)
Lung, Right							
Azygous lobe absent	9 (4.6)	8 (4.2)	13 (7.1)	2 (1.1)	0	6 (3.2)	6 (2.8)
Subclavian Artery							
Retroesophageal	0	1 (0.5)	0	0	0	0	0
Thoracic Cavity							

Fluid Filled	0	0	0	0	0	0	1 (0.5)
Persistent truncus arteriosus	0	0	0	0	1 (0.5)	0	0
Summary of Visceral Obs.							
Total Malformations							
# Litters (%)	1 (4.5)	2 (9.5)	1 (5.0)	1 (5.0)	2 (9.5)	2 (9.1)	2 (9.1)
# Fetuses (%)	1 (0.5)	3 (1.6)	1 (0.5)	1 (0.6)	2 (1.0)	2 (1.1)	3 (1.4)
Total Variations							
# Litters (%)	6 (27.3)	8 (38.1)	8 (40.0)	4 (20.0)	2 (9.5)	8 (36.4)	9 (40.9)
# Fetuses (%)	10 (5.2)	11 (5.8)	14 (7.6)	4 (2.3)	2 (1.0)	9 (4.8)	14 (6.6)

There were no statistically significant increases in the total number of **skeletal malformations or variations** when examined on a litter basis. Statistical analysis on the basis of the number of fetuses examined was not completed by the sponsor. The table below reproduces the data in sponsor's table 11. Although there were several malformations noted which exceeded the incidence in the control group, these were not statistically significant. The incidence of most of these observations were within the historic control range for the laboratory or occurred in a single pup at a single dose group and therefore considered by the sponsor to be unrelated to the study drug. One fetus displayed multiple external and skeletal malformations, including sacral neural arches absent, fused or misaligned for sacral vertebrae, sternebrae absent, thoracic centra absent and/or fused and thoracic neural arches misaligned and/or misshapen. As these findings were in a single pup, not statistically significant and/or were within the historical control range, the sponsor does not consider them to be related to the test article.

Summary of Fetal Skeletal Observations
Incidence expressed as the number of fetuses affected (%)

Group	Vehicle	Lidocaine	Tetracaine	Lido/Tet
Dose (mg/kg)	**0**	**15**	**5**	**5/5**
# of Litters Evaluated	22	20	22	22
# of Fetuses Evaluated	194	176	187	212
Caudal vertebra(e)				
Neural arch(es) absent	0	0	0	1 (0.5)
Cervical vertebra(e)				
Centra, additional ossification	1 (0.5)	0	0	3 1.4)
Centra, misshapen	0	0	0	3 (1.4)
Neural arch(es), additional ossific.	4 (2.1)	0	5 (2.7)	5 (2.4)
Neural arch(es), misaligned	0	0	0	1 (0.5)
Neural arch(es), misshapen	0	0	0	1 (0.5)
Hindlimb				
Talus, not ossified	0	0	0	2 (0.9)
Rib(s)				
Rib(s), discontinuous	1 (0.5)	0	0	1 (0.5)
Rib(s), fused	0	0	0	1 (0.5)
Rib(s), rudimentary	50 (25.8)	48 (27.3)	53 (28.3)	60 (28.3)
Rib(s), unilateral fill rib	28 (14.4)	26 (14.8)	20 (10.7)	34 (16.0)
Sacral Vertebra(e)				
Neural arch(es), absent	0	0	0	1 (0.5)
Neural arch(es), fused	0	0	0	1 (0.5)
Neural arch(es), misaligned	0	0	0	1 (0.5)
Skull				
Frontal bone, additional ossific.	0	0	3 (1.6)	1 (0.5)
Hyoid arch, bent	9 (4.6)	11 (6.3)	7 (3.7)	8 (3.8)

Hyoid body, not ossified	1 (0.5)	2 (1.1)	0	3 (1.4)
Nasal bone, additional ossific.	0	0	0	1 (0.5)
Sternum				
Sternebra(e), absent	0	0	0	1 (0.5)
Sternebra(e), additional ossific.	4 (2.1)	6 (3.4)	3 (1.6)	2 (0.9)
Sternebra(e), fused	2 (1.0)	6 (3.4)	2 (1.1)	2 (0.9)
Sternebra(e), misaligned	2 (1.0)	1 (0.6)	0	0
Sternebra(e), not ossified	16 (8.2)	16 (9.1)	20 (10.7)	32 (15.1)
Thoracic vertebra(e)				
Centra, absent	0	0	0	1 (0.5)
Centra, fused	0	0	0	1 (0.5)
Neural arch(es), misaligned	0	0	0	1 (0.5)
Neural arch(es), misshapen	0	0	0	1 (0.5)
Summary of Skeletal Obs.				
Total Malformations				
# Litters (%)	3 (13.6)	5 (25)	2 (9.1)	5 (22.7)
# Fetuses (%)	3 (1.5)	6 (3.4)	2 (1.1)	7 (3.3)
Total Variations				
# Litters (%)	22 (100)	20 (100)	20 (90.9)	22 (100)
# Fetuses (%)	80 (41.2)	80 (45.4)	78 (41.7)	99 (46.7)

Closer inspection of the individual fetus findings for the rare abnormalities noted above, indicates that fetus 9 from dam 346 who was treated with 5 /5 mg/kd lidocaine/tetracaine presented with the following malformations and variations:

Malformations and Variations in Fetus 9 from Dam 346 (lidocaine/tetracaine treatment group)			
Area	**Location**	**Classification**	**Observation**
External Observations			
Body	Abdomen	Malformation	Gastroschisis
Forelimb(s)	Digits	Malformation	Ectrodactyly
Forelimb(s)	Entire	Variation	Abnormal flexure
Hind limb(s)	Entire	Variation	Abnormal flexure
Hind limb(s)	Entire	Malformation	Malrotate
Tail	Entire	Malformation	Absent
Visceral Observations			
Abdominal cavity	Gallbladder	Variation	Smaller than normal
Abdominal cavity	Kidney	Malformation	Absent
Abdominal cavity	Ureter	Malformation	Absent
Skeletal Observations			
Thoracic vertebra(e)	Centra	Malformation	Absent
Thoracic vertebra(e)	Centra	Malformation	Fused
Skull	Hyoid body	Variation	Not ossified
Caudal vertebra(e)	Neural arch(es)	Malformation	Absent
Sacral vertebra(e)	Neural arch(es)	Malformation	Absent
Sacral vertebra(e)	Neural arch(es)	Malformation	Fused
Sacral vertebra(e)	Neural arch(es)	Malformation	Misaligned
Thoracic vertebra(e)	Neural arch(es)	Malformation	Misaligned
Thoracic vertebra(e)	Neural arch(es)	Malformation	Misshapen
Ribs	Ribs	Malformation	Fused
Sternum	Sternebra(e)	Variation	Absent
Sternum	Sternebra(e)	Variation	Not ossified
Hind limb(s)	talus	Variation	Not ossified

Dam 346 had a low final body weight and adjusted final body weight 2.964 kg. In addition, fetus 9 was approximately 15 grams less mass than the other 10 fetuses in the litter and clearly stands out from the remainder of the fetuses. Collectively, these data are not consistent with a teratogenic effect of lidocaine or tetracaine. The evidence for material toxicity in this Dam is a more likely explanation of the fetal effects noted for a good sized litter. This reviewer does not feel that the finding is related to the drug-treatment.

3.4.7 Local tolerance

The local tolerance studies submitted with the NDA were originally reviewed by Dr. Kathleen Haberny for IND 58,823 (Original IND, Submitted July 26, 1999). To complete the review of the NDA, Dr. Haberny's review was transformed into the new NDA format. However, my interpretations of the study results are in total agreement with the Dr. Haberny (and therefore are verbatim at times).

Study title: Modified Primary Skin Irritation Test

Key study findings: Based on Mean Primary Irritation (MPI) scores, the test patch and the mineral oil control were considered to be mildly irritating to the skin of the test animals. In contrast, the placebo patch was considered to be non-irritating to the skin.

Study no.:	NV X9C009G
Volume #, and page #:	Volume 10, Page 1
Conducting laboratory and location:	[redacted]
Date of study initiation:	March 2, 1999
GLP compliance:	Yes
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	Active S-Caine™ Local Anesthetic Patch with CHADD (Controlled Heat), Lot IP 02-24-99. This is developmental formulation A, **not the final formulation**. This [redacted]
Formulation/vehicle:	S-Caine™ Local Anesthetic Patch with CHADD (Controlled Heat), Lot PL 02-24-99

Methods

Doses: The rabbits were presumably with the clinical formation and therefore were exposed to patches containing 70 mg of lidocaine and 70 mg of tetracaine.

Study design: Six New Zealand White rabbits were used for the experiment (2.2-2.9 kg, young adult, female). One day prior to dosing the hair on the back of the animal is removed with clippers. Prior to dose administration, approximately 3 mls of blood was collected to serve as a baseline for subsequent samples to be used for toxicokinetic analysis (samples obtained both 1 and 2 hours after drug treatment). Blood samples were obtained from the auricular artery. Neostigmine was added to the collection tubes to prevent the plasma hydrolysis of tetracaine. Plasma samples were analyzed via the TDxFLx system that uses fluorescence polarized immunoassay technology. The limit of quantitation was 100 ng/ml.

Three dosing sites are used per animal as depicted in the diagram below:

Head
Site A Site B
Site C
Tail

Placement of test and control articles were randomized. The test articles (active S-Caine™ patch or placebo S-Caine™ patch) and control material (mineral oil) are applied to the intact skin of each animal for 1 hour. The test article was gently removed and any residual materials were removed by gently wiping the test site with gauze soaked in water. The sites were observed immediately after removal of the test and control materials and at approximately 2, 12, 24, 48, 72 and 96 hours. Edema, erythema and/or eschar formation were scored. A separate score was made for each site and a mean primary irritation score was calculated.

The Mean Primary Irritation (MPI) score for each test and control material was calculated as follows: For each observation period, the average erythema score was calculated by dividing the sum of the erythema scores for all 6 animals by 6. The edema scores were calculated in the same manner. The sum of the 24, 48 and 72 hour average erythema and edema scores was divided by 3 to obtain the MPI score.

Skin reactions were evaluated by the method of Draize with the following scale.

Evaluation of Skin Reactions[1]

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised about 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

1. From 16 CFR Part 1500.41, 1995.

Table 6
Descriptive Rating for Mean Primary Irritation Score[2]

Range of Values	Descriptive Rating
0	Non-irritating
> 0 - 2	Mildly irritating
> 2 - 5	Moderately irritating
≥ 6	Severely irritating

2. Draize J.H. "The Appraisal of Chemicals in Food, Drugs, and Cosmetics." Dermal Toxicity, pp. 45-59. Association of Food and Drug Officials of the United States, Topeka, Kansas (1965).

Results: All animals remained healthy during the study.

The test patch produced a very slight erythema at 12 hours in 3 of 6 animals. At the 24, 48 and 72 hour time points, 2 of 6 animals presented with very slight erythema. At the 96 hour time point, all irritation was completely reversed. No edema was noted at any time point.

The Placebo patch demonstrated no erythema or edema at any time point. The mineral oil control treatment produced a very slight erythema in 1 of 6 rabbits at 24 hours. There were no observations of edema.

Based upon these data, the test patch and the mineral oil could be considered to be mildly irritating to the skin. The placebo patch appears to be non-irritating to the skin.

The table below summarizes the skin scores at 24, 48 and 72 hours:

Table 8
Primary Dermal Irritation Scores (+24, +48 and +72 hrs)

Sample:	1 (test)			2 (placebo)			3 (mineral oil)		
Time/hr:	24	48	72	24	48	72	24	48	72
Rabbit No. 22627									
Erythema	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0
Rabbit No. 22694									
Erythema	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0
Rabbit No. 22700									
Erythema	1	1	1	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0
Rabbit No. 22703									
Erythema	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0
Rabbit No. 22704									
Erythema	0	0	0	0	0	0	1	0	0
Edema	0	0	0	0	0	0	0	0	0
Rabbit No. 22705									
Erythema	1	1	1	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0
Erythema Totals:	2	2	2	0	0	0	1	0	0
Edema Totals:	0	0	0	0	0	0	0	0	0
Erythema Average:		1			0			0.2	
Edema Average:		0			0			0	
MPI		0.3			0			0.1	

Toxicokinetics: Lidocaine (0.12 μg/ml) was detected in one of the 18 plasma samples, at 1 hour after patch application. Tetracaine was found in 5 of the 18 samples; 3 samples at 1 hour after patch application (6.08, 5.46 and 13.66 ng/ml) and 2 at 2 hours after application (6.15 and 5.28 ng/ml). The sponsor's Table 1 below presents each animals results.

Table 1. Plasma Concentrations of Lidocaine and Tetracaine in Female Rabbits with One S-Caine™ Patch Applied for One Hour

	Lidocaine (ng/mL)			Tetracaine (ng/mL)		
Rabbit No.	Predose	1 hr	2 hr	Predose	1 hr	2 hr
22627	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
22694	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
22700	BLQ	BLQ	BLQ	BLQ	6.08	6.15
22703	BLQ	BLQ	BLQ	BLQ	5.46	BLQ
22704	BLQ	120	BLQ	BLQ	13.66	BLQ
22705	BLQ	BLQ	BLQ	BLQ	BLQ	5.28
Mean assuming BLQ = 0						
Mean	0	20	0	0	4.20	1.91
Mean assuming BLQ = 0.5 LOQ						
Mean		62	50		5.45	3.57

BLQ = Below the limit of quantitation; 100 ng/mL for lidocaine and 5 ng/mL for tetracaine

Study title: A Dermal Irritation Study of S-Caine™ Patch in Rabbits

Key study findings: A single application of the final formulation of the S-Caine™ patch to the dorsal surface of male rabbits for one hour produced very mild irritation. In addition, plasma levels of both lidocaine and tetracaine were measurable with a T_{max} of about 2 hours. The C_{max} and $AUC_{0\text{-}24h}$ values for lidocaine are over 10-fold higher than those of tetracaine. For lidocaine the highest C_{max} was 194 ng/ml or ~1/25th the plasma levels considered to be potentially toxic to adult humans. In contrast, the highest C_{max} value for tetracaine was 13 ng/ml.

Study no.: 925-002
Volume #, and page #: Volume 10, Page 24
Conducting laboratory and location: [redacted]

Date of study initiation: November 12, 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: S-Caine™ Patch, Lot No. 1262
Formulation/vehicle: Placebo Patch. The final clinical formulation was used for this study.

Methods

Doses: Test article was applied dermally once on Day 1. Patch contains 70 mg lidocaine and 70 mg tetracaine.

Study design: Four male New Zealand White Hra (NZW)SPF rabbits, male. Body weights ranged from 2.87 to 3.06 kg. Animals were randomized to two groups (3 in one

group and the fourth animal in the other group). One day prior to administration, one exposure site on each rabbit was prepared for treatment by clipping the hair on the dorsal region. The test site was not abraded. Rabbits received the test article once by dermal application. During dosing it was determined that the test article patch would not stick to the skin properly, so the keep it in contact with the skin, the rabbit was wrapped around the dose site with four-ply gauze and [redacted] tape. Elizabethan-type collars were also applied and remained on each animal for the one-hour treatment time. The test site of the back of each animal was evaluated for erythema and edema according to the skin reaction scale below. Evaluations were conducted at the time of patch removal and at 24, 48 and 72 hours following patch application.

Mortality and Cageside Observations: Animals were checked twice each day throughout the duration of the study.

Body Weights: Body weights were recorded at arrival, on the day prior to the test article application and prior to test article application.

Dermal Irritation Evaluations: The test site was evaluated for erythema and edema according to the skin reaction scale given below at the time of patch removal and at 24, 48 and 72 hours following patch removal. This is based on a Draize scale for scoring skin irritation.

Erythema and Eschar Formation	
Score	Observation
0	No erythema
1	Very slight erythema (barely perceptible)
2	Well-defined erythema
3	Moderate to severe erythema
4	Severe erythema (beet redness) to slight eschar formation (injuries in depth)
	Maximum possible score = 4

Edema Formation	
Score	Observation
0	No edema
1	Very slight edema (barely perceptible)
2	Slight edema (edges of area well-defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)
	Maximum possible score = 4

The Mean Primary Irritation Index (PII) is the average erythema score at 24 and 72 hours after patch removal plus the average edema score at 24 and 72 hours after patch application. A descriptive rating was provided by the sponsor and reproduced below:

Mean Primary Irritation Index	
Descriptive Rating	Range of Index Values
Non-Irritating	0
Mildly Irritating	0.1 - 2
Moderately Irritating	2.1 - 5
Severely Irritating	5.1 - 8

Plasma Analysis: Blood samples were collected from the central ear artery of all animals. Samples were collected prior to patch application and at 1, 2, 6 and 24 hours after patch application on Day 1. The blood was placed in a tube containing potassium EDTA as an anticoagulant

Post-mortem evaluations: At study day 8, animals were euthanized via intravenous overdose with sodium pentobarbital. The carcass was discarded without necropsy.

Results: There were no mortalities and no evidence of irritation observed in any rabbit at the time of patch removal. At 24 hours, very slight to well-defined erythema was observed in all three test rabbits. Two rabbits had a very slight erythema at 48 hours. There was no evidence of irritation in any rabbit at 72 hours. Edema was not observed at any time point. The MPI index was 1.3, indicating that the patch was mildly irritating.

Application of the S-Caine™ patch to the intact skin of the male rabbit for one hour resulted in absorption of both lidocaine and tetracaine. Plasma levels of lidocaine were approximately 10-times higher than tetracaine. Systemic exposure, as measured by $AUC_{0\text{-}24h}$ was also ten times higher for lidocaine than for tetracaine. There was some evidence that both drugs were stored in a depot in the skin that is released slowly over time.

Figure 1. Plasma Concentrations of Lidocaine for Male Rabbits with One S-Caine Patch Applied for One Hour

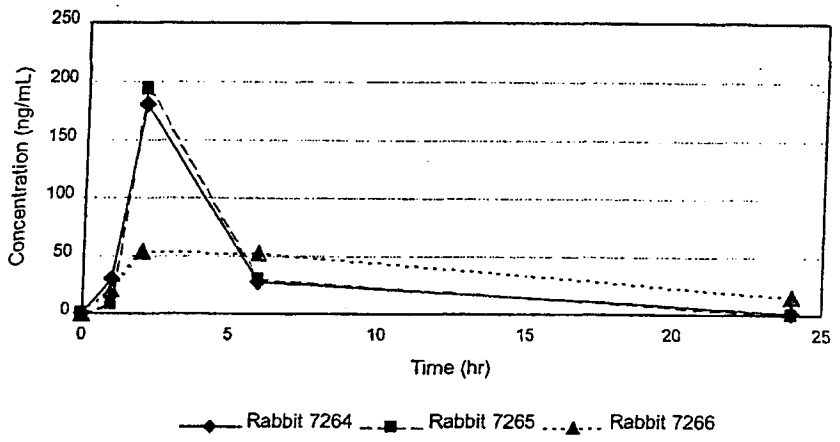
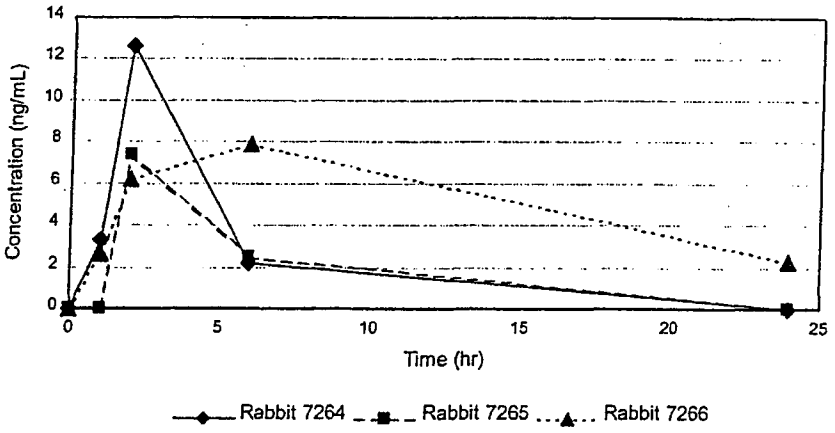


Figure 2. Plasma Concentrations of Tetracaine for Male Rabbits with One S-Caine Patch Applied for One Hour



The plasma concentrations of lidocaine and tetracaine in the male rabbit with S-Caine™ Patch are depicted graphically above. The plasma concentrations, C_{max}, $AUC_{0\text{-}24h}$ and T_{max} are listed in the tables below. From the data, it is clear that mean C_{max} of lidocaine are almost ~15 times as high as tetracaine and the AUC is over 10 times that of tetracaine.

Table 1. Plasma Concentrations of Lidocaine and Tetracaine in Male Rabbits with 1 S-Caine™ Patch Applied for One Hour

Time (hr)	Lidocaine Concentration (ng/mL)				Tetracaine Concentration (ng/mL)			
	Rabbit 7264	Rabbit 7265	Rabbit 7266	Mean ± SD	Rabbit 7264	Rabbit 7265	Rabbit 7266	Mean ± SD
0	BLQ	BLQ	BLQ	0	BLQ	BLQ	BLQ	0
1	30.76	7.54	20.72	19.7 ± 11.6	3.25	BLQ	2.62	2.0 ± 1.7
2	179.28	193.60	54.51	142.5 ± 76.5	12.59	7.36	6.18	8.7 ± 3.4
6	27.16	30.63	53.15	37.0 ± 14.1	2.22	2.46	7.91	4.2 ± 3.2
24	1.32	BLQ	16.10	5.8 ± 8.9	BLQ	BLQ	2.23	0.7 ± 1.3

BLQ = below the limit of quantitation, 0.9 ng/mL.

Table 2. Pharmacokinetic Parameters for Male Rabbits with One S-Caine Patch Applied for One Hour

Parameter	Lidocaine				Tetracaine			
	Rabbit 7264	Rabbit 7265	Rabbit 7266	Mean ± SD	Rabbit 7264	Rabbit 7265	Rabbit 7266	Mean ± SD
C_{max} (ng/mL)	179.3	193.6	54.5	142.5 ± 76.5	12.6	7.4	7.9	9.3 ± 2.9
T_{max} (ng/mL)	2	2	2	2.0 ± 0.0	2	2	6	3.3 ± 2.3
AUC_{0-24} (ng•hr/mL)	790	828	887	835 ± 49	59	45	125	77 ± 43

Study title: Dermal Absorption and Dermal Irritation Study of S-Caine™ Patch in Neonatal Piglets (with Toxicokinetics)

Key study findings: Neonatal piglets were treated with the S-Caine™ Patch, Placebo S-Caine™ Patch or mineral oil. Local dermal irritation potential and plasma levels of lidocaine and tetracaine were examined. There were no differences between the placebo-patch and the innovator patch, indicating minimal effects of the S-Caine™ patch to produce a local tissue reaction under the conditions tested.

Study no.: 925-003
Volume #, and page #: Volume 10, Page 89
Conducting laboratory and location: [redacted]
Date of study initiation: November 7, 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: S-Caine™ Patch, Lot No. 1262
This is the **final clinical formulation** of the S-Caine™ Patch.
Formulation/vehicle: Placebo S-Caine™ Patch, Lot No. 1264.
Mineral oil was used as a control article.

The placebo S-Caine™ Patch contains the exact same components as the S-Caine™ Patch except the lidocaine and tetracaine were replaced by olive oil.

Methods

Doses: Single application of the S-Caine™ Patch (70 mg lidocaine and 70 mg tetracaine).

Group Assignment			
Group	Dose Interval (minutes)	Number of Animals	
		Male	Female
1	30	3	3
2	60	3	3

Study design: The study examined the dermal absorption and potential dermal irritant effect of the S-Caine™ patch in neonatal piglets (N=6/sex, approximately 1 day old at dosing). Specifically, the piglets were Landrace-Duroc Cross bred swine. The piglets were exposed to test article (S-Caine™ Patch), Placebo (S-Caine™ Placebo Patch) and a control article (mineral oil). The patches were applied to intact dorsal skin. Group 1 (3 males and 3 females) were exposed for 30 minutes and Group 2 (3 males and 3 females) were exposed to 60 minutes. Of note, the S-Caine™ patch used in the piglet study was the same patch size recommended for use in children.

Animals were observed at least twice daily for morbidity, mortality, injury and availability of food and water. Clinical observations were conducted daily. Individual body weights were measured and recorded prior to test article administration and at study termination. Each of the three sites were assessed for irritation at 1, 24, 48 and 72 hours following patch removal and scored using a standard Draize scale (see below).

4.3.4.1. Erythema and Eschar Formation

Erythema Score	Clinical Description
0	No erythema
1	Very slight erythema (barely perceptible)
2	Well-defined erythema
3	Moderate to severe erythema
4	Severe erythema (beet redness) to slight eschar formation (injuries in depth)
Maximum possible = 4	

4.3.4.2. Edema Formation

Edema Score	Clinical Description
0	No edema
1	Very slight edema (barely perceptible)
2	Slight edema (edges of area well defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure
Maximum possible = 4	

Blood samples were collected at 0, 1, 2, 3, 4 and 5 hours following patch application. A microscopic evaluation of each of the test sites and an untreated site for each animal was conducted. Blood was collected into vacutainers containing potassium EDTA as an anticoagulant and neostigmine as a cholinesterase inhibitor. The determination of the lidocaine and tetracaine in the samples was completed simultaneously using a gas chromatography method with nitrogen phosphorus detection. For lidocaine the limit of quantitation was 100 ng/ml and the linear range was 100 to 2000 ng/ml. For tetracaine, the limit was 5 ng/ml. The linear range was 100 to 2000 ng/ml. For tetracaine, the limit of quantitation was 5 ng/ml and the range was 5 to 200 ng/ml.

Necroscopic examinations were performed on all animals following 72-hour post-exposure evaluation. Euthanasia was completed via sodium pentobarbital administration. Sections of skin from each treatment site and an untreated site were collected for histological analysis. Skin sections were stained with hematoxylin and eosin. Following the skin sample collection, the animals were discarded without further evaluation.

Results: There were no mortalities in this study. Clinical observations noted abrasions on the skin on 2 observations between days 1 and 4 that were restricted to one of the males treated for 60 minutes with S-Caine™ Patch. There were no abrasions noted in the females. There were not differences in body weights values between treatment groups.

There were no signs of dermal irritation in any treatment group at any time point examined in this study. The pharmacokinetics analysis indicates that lidocaine is absorbed from the S-Caine™ patch and produces low plasma levels. However, levels

were not detected in all animals. The results also suggest that lidocaine can be absorbed into the skin to produce a depot

Table 2. Pharmacokinetic Parameters for Neonatal Piglets Receiving S-Caine™ Topically

Piglet No.	Application Time (min)	Sex	Weight Day 1 (kg)	Lidocaine				Tetracaine			
				C_{max} (ng/mL)	T_{max} (hr)	Minimum AUC_{0-5} (ng•hr/mL)	Maximum AUC_{0-5} (ng•hr/mL)	C_{max} (ng/mL)	T_{max} (hr)	Minimum AUC_{0-5} (ng•hr/mL)	Maximum AUC_{0-5} (ng•hr/mL)
7333	30	M	1.60	< 200	*	260	660	< 10	*	0	28
7334	30	M	2.07	< 200	*	345	665	< 10	*	0	26
7335	30	M	2.05	< 200	*	180	500	< 10	*	0	22
Means			1.91	< 200		262	608	< 10		0	25.3
7339	30	F	2.15	< 200	*	0	480	< 10	*	0	24
7340	30	F	1.85	< 250	*	240	680	15.0	4	35.2	41.2
7341	30	F	1.62	260	3	610	970	< 12.5	*	21.3	39.3
Means			1.87	237		283	710	12.5		18.9	34.9
7336	60	M	1.72	280	5	640	720	< 5	*	0	18
7337	60	M	2.16	420	5	1500	1500	< 10	*	0	22
7338	60	M	1.80	<250	*	0	720	< 12.5	*	6.2	42.2
Means			1.89	317		713	980	< 9.2		2.1	27.4
7342	60	F	1.91	< 200	*	305	625	< 10	*	0	26
7343	60	F	1.71	240	2	480	720	< 10	*	0	26
7344	60	F	1.81	230	2	315	715	< 10	*	0	30
Means			1.81	223		367	687	< 10		0	27.3

* For C_{max} values below the limit of quantitation, it is difficult to assign a value for T_{max} because there is more than one sampling time with possible C_{max} concentrations.

3.4.8 Special toxicology studies

Dermal Sensitization – Beuhler Method

[redacted]

March 8 – May 20, 1999. GLP and Quality Assurance statements: signed and present.

Methods: This study was conducted according to the methods of Buehler (1965, Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermatol. 91:171). Adult male guinea pigs (Hartley albino, [redacted] weights 571-665 g, n=20 test, 10 test naïve, 5 positive control and 5 naïve positive control) received 3 topical applications of the test patches (S-Caine™ Local Anesthetic Patch with CHADD (Controlled Heat) Patch, ZARS, Inc., Lot #s IP-02-19-99, IP-02-20-99, IP-02-24-99, IP-4-20-99 and IP-44-13-99, 1:1 eutectic drug mixture of lidocaine base and tetracaine base, 1.5" x 2.125", 1/16" thick drug patch, 3/16" thick CHADD patch) or control patches on shaved skin sites on the right side for 6 hours each on days 0, 7 and 14. The positive control was dinitrochlorobenzene (DNCB, [redacted] Lot # 64H0790, 9.5% in ethanol on [redacted] of 25 mm diameter and Lot # 10XBA2012). The negative control was Placebo S-Caine™ Local Anesthetic with CHADD (Controlled Heat, ZARS, Inc.). Fourteen days after the 3rd exposure, the guinea pigs were challenged with topical applications of the test and positive control patches for 6 hours on a previously unexposed shaved area on the left side. The application sites were examined and scored for erythema and edema 24 hours after each induction and challenge exposure and 48 hours after the challenge exposure. The application sites were scored as follows: 0 = no reaction; 0.5 = slight patchy erythema; 1 = slight confluent or moderate patchy erythema; 2 = moderate erythema; 3 = erythema, edema and cracking of the skin.

Results:

	Number of Guinea Pigs with Reaction				
Score	0	0.5	1	2	3
Test Article (n=20)					
1st Exposure	15	5	0	0	0
2nd Exposure	0	5	15	0	0
3rd Exposure	0	3	13	4	0
Challenge (24h)	5	8	4	3	0
(48h)	5	11	4	0	0
Test Naive (n=10)					
1st Exposure	NA*	NA	NA	NA	NA
2nd Exposure	NA	NA	NA	NA	NA
3rd Exposure	NA	NA	NA	NA	NA
Challenge	0	4	0	0	0
(48h)	5	5	0	0	0
Positive Control (n=-5)					
1st Exposure	0	5	0	0	0
2nd Exposure	0	5	0	0	0
3rd Exposure	0	5	0	0	0
Challenge (24h)	0	0	1	4	0
(48h)	0	0	1	4	0
Positive Naive Control (n=-5)					
1st Exposure	NA	NA	NA	NA	NA
2nd Exposure	NA	NA	NA	NA	NA
3rd Exposure	NA	NA	NA	NA	NA
Challenge (24h)	5	0	0	0	0
(48h)	5	0	0	0	0

*NA: Not applicable. Animals in these groups did not receive induction phase exposure.

The incidence scores (number of animals in each group showing a score of 1 or higher, divided by the total number of animals in the group) were 0.4 and 0.2 in the test group at 24 and 48 hours respectively and 1 in the positive control group at both timepoints. The severity index (sum of test scores divided by the total number of animals in the group) values were 0.6 and 0.5 in the test group at 24 and 48 hours respectively, 0.2 and 0.3 in the test naïve group, 1.8 at both timepoints in the positive control group and 0 at both timepoints in the positive naïve control group.

Conclusions: The results of this study showed that the test article, S-Caine™ Patch + CHADD Patch induced sensitization in guinea pigs with less intensity than the positive control DNCB, and is a potential sensitizer in humans. The S-Caine™ and CHADD patches were not individually tested. The quantitative composition of the patches was not provided in the report, and will be requested from the sponsor.

CHADD™ Patch Skin Temperature Test

Note: No information given on the performing laboratory, study dates, and GLP and Quality Assurance compliance.

Methods: No description of species, test article (e.g., source, Lot #s), methods for measurement of skin temperature, or other information was provided. Twelve CHADD patches were applied to the skin of an unknown animal(s) or volunteer(s), and skin temperature was measured for 30 minutes.

Results: Mean skin temperature was 38°C and 39.5°C at 15 and 30 minutes respectively. The peak skin temperature was 41.2°C.

Conclusions: Interpretation of the results cannot be made without additional information on the methods. Therefore, the final study report will be requested. Evaluation of skin temperature

resulting from application of the final drug product (both S-Caine™ and CHADD patches) for a minimum of one hour application time will be recommended.

NOTE: At the time of the NDA filing, the CHADD Patch was determined to not provide any increase in efficacy or absorption in a clinical trial. This issue will be addressed via both CMC and clinical studies (RDM).

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The sponsor has completed acute and repeat-dose toxicity studies focusing on the potential for local tissue irritation. In addition, the standard battery of genetic toxicology studies was completed for both lidocaine and tetracaine. The results of the genetic toxicology studies for lidocaine are consistent with other reports available to the Agency. The genetic toxicology studies on tetracaine are the first to be completed to my knowledge. Under the conditions of the assay, tetracaine base was negative in the in vitro bacterial reverse mutation assay (Ames assay) and the *in vivo* mouse micronucleus assay. Tetracaine, however, tested equivocal in the *in vitro* chromosome aberration assay in CHO cells in the presence of metabolic activation.

The sponsor examined the effects of lidocaine, tetracaine and the combination of the two drugs on the embryofetal development (Segment II studies) in both rats and rabbits. The results of the studies indicate that lidocaine, tetracaine or the combination of the two (under the conditions tested) were not teratogenic. The single rabbit fetus (number 9 from Dam 346) that contained a large number of malformations and variations appears to be the result of maternal stress and crowding in the uterus. This fetus had a lower fetal weight than its litter mates and was born to a dam that was clearly lighter than the rest of the animals in the group. These observations are not consistent with a teratogenic effect of either lidocaine:tetracaine but are more likely due to maternal stress and crowding in the uterus.

Unresolved toxicology issues (if any):

There are three unresolved toxicology issues noted in this NDA submission as follows:

1) The Division made an agreement with the sponsor that the Segment I and Segment III reproductive toxicology studies on tetracaine would be completed and submitted as a phase 4 agreement if the NDA was approved on the first cycle. However, if the NDA is not approved on the first cycle, the studies should be submitted with the second cycle submission.

2) The equivocal finding for tetracaine in the in vitro chromosome aberration assay should be followed-up by the sponsor. Given the high degree of cytotoxicity in the positive culture (85%), it is possible that this may be due to an artifact. The sponsor should repeat this study and characterize the culture conditions to determine if the positive finding could be attributed to pH or osmolarity changes or if the finding replicates. This should be completed for the second cycle submission.

3) The sponsor is relying upon the published literature to fulfill the requirements for reproductive toxicology studies for lidocaine, specifically Segment I and Segment III studies. During the pre-NDA meeting, the sponsor was informed that "NDA submission should include the referenced reproduction toxicity studies with lidocaine along with the sponsor's assessment of the adequacy of these studies according to current standards (e.g., dose selection, dose regimen)." Although the sponsor has provided a summary of published reports, there was no discussion of the adequacy of the studies according to current standards. Further, the study reports were not provided with the NDA submission to allow for detailed critical review. Finally, there appears to be significant portions of the current standard segment I study not addressed by the literature references provided. For example, there are no data describing the effects of lidocaine treatment of the male prior to mating (generally 4 week pretreatment is recommended). As a result, there are no descriptions or discussions of the potential effect of local anesthetics on sperm morphology or mobility. The dosing rationale was not explained in the summary. The sponsor should critically review the existing study reports and provide a complete description of the potential effects of lidocaine on fertility and early embryonic development. This is particularly important in light of the summarized study reports suggesting early embryonic effects following a single dose of lidocaine. These findings were not addressed by the sponsor.

Recommendations: From the non-clinical pharmacology and toxicology perspective, the drug product, S-Caine™ Patch, is deemed **approvable**. Prior to approval, however, the sponsor should address the above deficiencies and provided data (literature based or experimental if published literature is not available) to completely characterize the issues of genetic toxicology and reproductive toxicology of lidocaine.

Suggested labeling: The proposed labeling as presented in the NDA supplement is listed below:

Carcinogenesis, Mutagenesis, Impairment of Fertility:

Carcinogenesis:

Mutagenesis: The mutagenic potential of lidocaine base and tetracaine base has been determined in the Ames Bacterial Reverse Mutation Assay, the *in vitro* chromosomal aberration assay using Chinese hamster ovary cells, and the *in vivo* mouse micronucleus assay. Lidocaine was negative in all three assays. Tetracaine was negative in the Ames assay and the mouse micronucleus assay. Mixed results were obtained for tetracaine in the *in vitro* chromosomal aberration assay. Tetracaine was negative in the absence of metabolic activation, and equivocal in the presence of metabolic activation.

Impairment of Fertility:

[redacted]

Use in Pregnancy:

Teratogenic Effects: Pregnancy Category B.

[redacted]

Signatures (optional):

Reviewer Signature ____R. Daniel Mellon, Ph.D._____

Supervisor Signature_____R. Daniel Mellon, Ph.D._____ Concurrence Yes _X_ No ___

3.7. APPENDIX/ATTACHMENTS

Reference List

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/s/

R. Daniel Mellon
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