

**2.6.6.4 Genetic toxicology** These studies were previously reviewed by the first reviewer for the IND. They were given a secondary review here for the sake of completeness.

**Study title:** Ames test-reverse mutation assay on *Salmonella typhimurium*

**Key findings:** Toxicity was seen  $\geq 50$   $\mu\text{g}/\text{plate}$  (-S9) and  $\geq 250$   $\mu\text{g}/\text{plate}$  (+S9). Under the conditions of the study an increase in revertants was not seen. A decrease in revertants was apparent both  $\pm$ S9.

**Study no.:** CEL0593

**Conducting laboratory and location:** Sanofi Dept of Toxicology , Montpellier Cedex, France

**Date of study initiation:** May 14, 1991

**GLP compliance:** statement included

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** SR335898 batch MR 13-171-1 dissolved in DMSO, purity by LC 99.6%

**Methods** *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, TA102 were used  $\pm$ S9 activation. A pre-incubation method was used in this study: test tubes were incubated for 20 minutes at 37°C before addition of the top agar.

A preliminary bacterial toxicity test was performed with and without metabolic activation, on tester strain TA98, with a top concentration of 5000  $\mu\text{g}/\text{plate}$ . According to these results, the genotoxicity test (CEL593) was carried out at 5 decreasing concentrations of SR 33589B on 5 *Salmonella typhimurium* tester strains.

Test	Concentrations used $\mu\text{g}/\text{plate}$ (conversion factor of 1.066 for salified )
<b>Toxicity test (TA98 only)</b>	
Non-salified	94, 469, 938, 2345, 4690
Salified	100, 500, 1000, 2500, 5000
<b>Genotoxicity test</b>	
Non-salified	4.7, 9.4, 47, 94, 235
salified	5,10, 50, 100, 250
Positive controls -S9	Na azide, 2-nitrofluorene, 9-aminoacridine, mitomycin C
Positive controls+S9	2-aminoanthracene, danthron

**Results**

Toxic  $\geq 50$   $\mu\text{g}/\text{plate}$  -S9  
 $\geq 250$   $\mu\text{g}/\text{plate}$  +S9

A reverse dose response was apparent. That is, a decrease in the number of revertants was seen.

**Study title:** Ames Test-Reverse mutation assay on Salmonella typhimurium

**Key findings:** TA98 +S9 showed a repeatable increase in revertants. A full dose response was not shown due to the level of toxicity at the concentrations tested. Under the conditions of the assay, it appears that the test article in the presence of S9 activation caused an increase in revertants in the TA98 strain.

**Study no.:** Cel0709

**Conducting laboratory and location:** Sanofi Toxicology, Montpellier Cedex, France

**Date of study initiation:** March 23, 1993

**GLP compliance:** statement included

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** SR33589B batch 92-01, vehicle of DMSO

**Methods** Salmonella typhimurium tester strains TA1535, TA1537, TA102, TA98, TA100 were used  $\pm$ S9 in the plate incorporation method.

Test	Concentrations used $\mu\text{g}/\text{plate}$ (conversion factor of 1.066 for salified )
<b>Toxicity test (TA98 only)</b>	
Non-salified	4.7, 9.4, 47, 94, 235, 469
Salified	5,10, 50, 100, 250, 500
<b>Genotoxicity test</b>	
Non-salified	9.4, 23, 47, 94, 164
salified	10, 25, 50, 100, 175
Complementary study: TA98+S9	
Non-salified	9.4, 23, 47, 94, 164
salified	10, 25, 50, 100, 175
Positive controls -S9	Na azide, 2-nitrofluorene, 9-aminoacridine, mitomycin C
Positive controls+S9	2-aminoanthracene, danthron

**Results**

-S9: toxicity  $\geq 100 \mu\text{g/plate}$  except for TA100 (toxicity from  $\geq 175 \mu\text{g/plate}$ )

Residual toxicity at 25 and  $50 \mu\text{g/plate}$  in all other strains.

+S9: toxicity from  $\geq 100 \mu\text{g/plate}$  in all strains

An increase in revertants was seen -S9 at the  $50 \mu\text{g}$  concentration of TA98.

Table (3.1) 1.  
Bacterial toxicity on TA98 and TA100

Tester strain	Concentration ( $\mu\text{g/plate}$ )	Without S-9 mix		With S-9 mix	
		Bacterial <sup>(1)</sup> background lawn	Revertant <sup>(2)</sup> colonies/plate	Bacterial <sup>(1)</sup> background lawn	Revertant <sup>(2)</sup> colonies/plate
TA98	0	-	18	-	20
	5	-	19	-	14
	10	-	23	-	12
	50	-	21	-	42
	100	+	14	-	25
	250	++	5	++	0
	500	++	1	++	0
TA100	0	-	122	-	123
	5	-	123	-	120
	10	-	133	-	116
	50	-	114	-	121
	100	-	93	-	85
	250	-	48	-	105
	500	+	4	+	60

<sup>(1)</sup> Background lawn aspect  
 - Normal aspect  
 + Partial sparsity  
 ++ Sparsity  
<sup>(2)</sup> Mean of 3 plates for solvent control

Table (3.2.2) 1.  
AMES TEST : Group results (with S-9 mix)  
No. His+ revertant colonies/plate

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 : Strain :  
 : TA98 :  
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Group 0 : DMSO

: n = 5 :  
 : mean 28.4 :  
 : SEM 1.50 :

Group 1 : POSITIVE CONTROL

: n = 2 :  
 : mean 1540.0 :  
 : SEM 10.00 :

Group 2 : SR33589B 10 microg/plate

: n = 5 :  
 : mean 34.4 :  
 : SEM 2.79 :

Group 3 : SR33589B 25 microg/plate

: n = 5 :  
 : mean 37.2 :  
 : SEM 2.97 :  
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Table (3.2.2) 1  
 AMES TEST : Group results (with S-9 mix) (Cont'd)  
 No. His+ revertant colonies/plate

		Strain		
		TA98		
Group 4 :	SR33589B	50	microg/plate	
:	n = 5	:		:
:	mean	42.2	:	:
:	SEM	4.18	:	:
Group 5 :	SR33589B	75	microg/plate	
:	n = 5	:		:
:	mean	28.6	:	:
:	SEM	4.13	:	:
Group 6 :	SR33589B	100	microg/plate	
:	n = 5	:		:
:	mean	14.8	:	:
:	SEM	2.06	:	:
Group 7 :	SR33589B	175	microg/plate	
:	n = 3	:		:
:	mean	4.0	:	:
:	SEM	0.58	:	:

**Study title:** In vitro gene mutation assay at the locus TK +/- in mouse lymphoma L5178Y cells

**Key findings:** There was no indication of increased mutation frequency in the data as presented. The positive controls produced appropriate responses.

**Study no.:** FSRFU-LYM0125-EN-E01

**Conducting laboratory and location:** Sanofi-Synthelabo, Porcheville, France

**Date of study initiation:** June 25, 2002

**GLP compliance:** statement included

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** SR33589B, batch NFI 5659038, vehicle of DMSO

## Methods

### Summary of concentrations used.

Conditions	concentrations
3 hour exposure±S9	9.37, 18.75, 37.5, 75, 150, 300 µg/ml
24 hour exposure –S9	0.1, 0.25, 0.5, 1,2,4 and 5
1 <sup>st</sup> study: 3 hour exposure –S9	2.5,5,7.5,10, 12.5, 15
1 <sup>st</sup> study: 3 hour exposure +S9	10, 15,20, 25, 30 and 35
2 <sup>nd</sup> study: 24 hour exposure –S9	0.5, 1,1.5, 2, 2.5, and 3
2 <sup>nd</sup> study: 3 hour exposure +S9	17.5, 20, 22.5, 25, 27.5, 30
Positive control –S9	Methylmethane sulfonate (MMS)
Positive control +S9	cyclophosphamide

## Results

In the preliminary test, relative survival fell from 28% at 9.37µg/ml(-S9, 3 hours) to 0% at 18.75µg/ml under the same incubation conditions. With metabolic activation, relative survival decreased to ~5% at 37.5µg/ml with 3 hours incubation. In the 24 hour, -S9 preliminary test, relative survival at 2µg/ml was 8% and dropped to 0 at the next concentration of 4µg/ml.

There was no indication of increased mutation frequency in the data as presented. The positive controls produced appropriate responses.

**Study title:** In vitro gene mutation assay at the locus HPRT in Chinese Hamster V79 fibroblasts.

**Key findings:** With S9 activation, statistically significant increases in mutants were seen in three repeats of the assay.

**Study no.:**685-3-008

**Conducting laboratory and location:** Sanofi, Montpellier Cedex, France

**Date of study initiation:** March 19, 1993

**GLP compliance:** statement included

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:**SR33589B, batch 92.01, DMSO as vehicle

## Methods

V79 fibroblastic Chinese hamster cells were used  $\pm$ S9. The concentrations used are summarized below:

Summary of concentrations used

First study	$\pm$ S9	2.5,10,25, 50, 100 $\mu$ g/ml
Second study	-S9	1, 2.5, 5, 10, 17.5
	+S9	5, 10, 17.5, 25, 37.5

The highest concentration was based on the limit of solubility.

The positive controls used were

-S9: ethyl methane sulfonate (EMS)

+S9: benzo(a)pyrene [B(a)P].

## Results

Without S-9 mix, no gene mutation was induced at the locus HPRT in cultures treated with 2.5, 5 and 10  $\mu$ g/ml SR 33589B. At 25  $\mu$ g/ml, a statistically significant increase was recorded but since it was noted at a toxic concentration (only 27 % cell survival), it was not considered biologically relevant.

With S-9 mix, the mutation frequency values in cultures treated with 2.5, 5, 10 and 25  $\mu$ g/ml were in the same range as solvent control cultures whereas at 50  $\mu$ g/ml (corresponding to marked toxicity) no mutant colonies were seen.

HPRT/V79 test without metabolic activation

	cfeI	cfeII	Viable Cells X10 <sup>-6</sup>	Nb 6TG mutants	MF x 10 <sup>-6</sup>	Statistic p value
Untreated cells	1.18	1.21	4.37	13	2.98	0.73 ns
DMSO	0.97	1.14	4.11	14	3.41	
SR33589B 2.5 $\mu$ g/ml	0.91	1.23	4.42	0	0.00	<0.001***
SR33589B 5 $\mu$ g/ml	1.16	1.13	4.06	29	7.14	0.02*
SR33589B 10 $\mu$ g/ml	0.76	1.13	4.53	1	0.22	<0.001***
SR33589B 25 $\mu$ g/ml	0.26	1.07	4.29	71	16.54	<0.001***
EMS 3 mM	1.11	1.11	4.45	1093	245.43	<0.001***
EMS 6 mM	1.01	1.25	4.98	1932	387.95	<0.001***

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

## Second test: HPRT/V79 test without metabolic activation

	cfeI	cfeII	Viable Cells X10 <sup>-6</sup>	Nb 6TG mutants	MF x 10 <sup>-6</sup>	Statistic p value
Untreated cells	1.04	0.85	3.38	8	2.37	0.10 ns
DMSO	1.02	0.90	3.59	17	4.73	
SR33589B 1 µg/ml	1.07	0.88	3.53	4	1.13	0.005**
SR33589B 2.5 µg/ml	1.04	1.01	4.03	17	4.21	0.74 ns
SR33589B 5 µg/ml	1.03	0.98	3.91	46	11.77	0.001***
SR33589B 10 µg/ml	0.89	1.11	4.45	38	8.53	0.04
SR33589B 17.5 µg/ml	0.66	1.02	3.68	0	0.00	0.001***
EMS 3 mM	1.04	0.90	3.23	858	265.80	<0.001***
EMS 6 mM	1.00	1.00	3.98	1797	451.51	<0.001***

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

According to the sponsor:

With the S-9 mix

The mutation frequency was heterogeneous, with a statistically significant increase at 10-17.5 and 25 µg/ml, but concentration-unrelated and within historical control values. Therefore these responses have no biological relevance.

The high mutation frequency values obtained simultaneously for the positive controls (EMS and B(a)P proved that the cell system was potentially active.

## Second test: HPRT/V79 test with S9 activation

	cfeI	cfeII	Viable Cells X10 <sup>-6</sup>	Nb 6TG mutants	MF x 10 <sup>-6</sup>	Statistic p value
Untreated cells	1.02	1.01	4.03	5	1.24	0.31ns
DMSO	1.00	0.92	3.66	8	2.19	
SR33589B 5 µg/ml	0.96	0.98	3.90	16	4.10	0.14ns
SR33589B 10 µg/ml	0.96	0.97	3.87	35	9.05	<0.001***
SR33589B 17.5 µg/ml	0.89	0.98	3.93	41	10.44	<0.001***
SR33589B 25 µg/ml	0.90	1.03	4.10	33	8.05	<0.001***
SR33589B 37.5 µg/ml	0.34	0.94	3.77	4	1.06	0.23ns
BaP 10 µg/ml	0.81	1.02	3.65	197	53.91	<0.001***
BaP 20 µg/ml	1.15	1.00	4.01	255	63.54	<0.001***

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Although a dose response was observed, the sponsor dismissed the results for falling within the range of historical controls.

**Study title:** In vitro DNA repair assay on rat hepatocytes in primary culture

**Key findings:** SR33589B was cytotoxic at concentrations of 10 and 25 µg/ml. 5µg/ml had less toxicity but also killed cells. The cells treated with test compound showed results very similar to those of the cell and vehicle controls with respect to net nuclear count and % cells in repair. In this in vitro study there was no evidence of unscheduled DNA synthesis.

**Study no.:** DNA001

**Conducting laboratory and location:**

**Date of study initiation:** April 19, 1993

**GLP compliance:** statement included

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** SR33589B(batch92-01), vehicle of DMSO, purity 99.5%

### Methods

Primary culture hepatocytes were prepared from male Fischer rats. The concentrations used were 1, 2.5, 5, 10 and 25 µg/ml. Only the slides for 1, 2.5 and 5 µg/ml were read. DMSO was used as the vehicle.

**Results:** SR33589B was cytotoxic at concentrations of 10 and 25 µg/ml. 5µg/ml had less toxicity but also killed cells. The cells treated with test compound showed results very similar to those of the cell and vehicle controls with respect to net nuclear count and % cells in repair. In this in vitro study there was no evidence of unscheduled DNA synthesis. The positive control produced appropriate responses.

**Study title:** In vitro DNA repair assay on rat hepatocytes in primary culture

**Key findings:** SR33589B killed cells at 10 µg/ml with similar cell development as the controls. Substantial toxicity was seen at 25 µg/ml and precipitation ≥50µg/ml. Positive controls produced appropriate responses. Under the conditions of the assay unscheduled DNA synthesis was not seen following treatment with the test article.

**Study no.:** CEL0536

**Conducting laboratory and location:** Sanofi Research, Montpellier Cedex, France

**Date of study initiation:** May 15, 1991

**GLP compliance:** statement included

**QA reports:** yes ( ) no ( )

**Drug, lot #, and % purity:** SR33589B batch 13-171-11, purity; DMSO was used as the vehicle.

**Methods**

Primary hepatocyte cultures were prepared from male Fischer rats. The concentrations of test article used are summarized below.

Concentrations  $\mu\text{g/ml}$

Cytotoxicity study	1,5,10,100,250
DNA repair study	1,5,10,25
Slides for 5 and 10 $\mu\text{g/ml}$ were read	

Cells were washed after 2 hours of incubation. Immediately following washing, the cells were exposed to test compound or controls in the presence of  $10\mu\text{C/ml}$  for 18-20 hours.

**Results**

SR33589B killed cells at 10  $\mu\text{g/ml}$  with similar cell development as the controls. Substantial toxicity was seen at 25  $\mu\text{g/ml}$  and precipitation  $\geq 50\mu\text{g/ml}$ . Positive controls produced appropriate responses. Under the conditions of the assay unscheduled DNA synthesis was not seen following treatment with the test article.

**Study title:** Lymphocyte cytogenetic study

**Key findings:** An equivocal assay. There were some mild increases in aberrations that it would be easy to dismiss if the positive controls had given consistently robust responses.

**Study no.:** MAF0018

**Conducting laboratory and location:** Pharmaco L.S.R., Eye, Suffolk, England

**Date of study initiation:** March 14, 1994

**GLP compliance:** statement included

**QA reports:** yes (x) no ( )

**Drug, lot #, and % purity:** SR33589B batch 92.011-11, purity, DMSO as vehicle

**Methods**

Human peripheral blood lymphocytes were incubated with test compound to examine the effect on chromosomal structure. Tests were conducted  $\pm$ S9. Without the S9 mix cells were exposed continuously to test article for 24 to 48 hours. With S9, exposure was limited to 3 hours and cells were harvested 21 or 45 hours later. Colcemid was added to the preparations 3 hours prior to harvest. Mitotic indices were based on the number of metaphases observed per 1000 cells scored. Chromosome aberrations were scored by examination of 100 metaphases per culture.

## Summary of concentrations used

Concentrations used $\mu\text{g/ml}$	Preliminary dose-range finding assay	$\pm\text{S9}$ 24 and 48 hr sampling times: 25, 50, 100, 200, 600
	Main cytogenetic assay	-S9: 24 and 48 hrs: 1.25, 2.5, 5, 10, 15 and 20 +S9: 24hr sampling 6.25, 12.5, 25, 40 +S9: 48 hr 6.25, 12.5, 25, 35, 45
Doses used for cytogenetic analysis :duration of Tx +recovery in hours	-S9	24 $\pm$ 0: 1.25, 2.5, 5 $\mu\text{g/ml}$ 48 $\pm$ 0: 1.25, 2.5, 5 $\mu\text{g/ml}$
	+S9	3+21: 6.25, 12.5, 25 $\mu\text{g/ml}$ 3+45: 12.5, 25, 35 $\mu\text{g/ml}$
Metabolic activation	S9 from arochlor-induced rat liver	

Positive controls (duration of Treatment +recovery in hours      -S9: 24+0 and 48+0: Chlorambucil 2 $\mu\text{g/ml}$   
+S9: 3+21 and 3+45: cyclophosphamide 6 $\mu\text{g/ml}$ )

In the absence of S-9 mix, slides from all cultures treated with SR 335898 at 10, 15 or 20  $\mu\text{g/ml}$  were seen to contain few cells, and no metaphases. At the 24 hour sampling time, no toxicity (i.e. reduction in mitotic activity, compared to concurrent solvent controls) was apparent at 1.25  $\mu\text{g/ml}$ : reductions in mitotic activity of 29 and 65% were seen at 2.5 and 5.0  $\mu\text{g/ml}$  respectively. At the 48 hour sampling time, no real toxicity was seen at 1.25  $\mu\text{g/ml}$ : reductions in mitotic activity of 23 and 82% were seen at 2.5 and 5.0  $\mu\text{g/ml}$ . Thus dose-related toxicity was apparent at both sampling times.

In the presence of S-9 mix, at the 24 hour sampling time dose-related toxicity was apparent, with reductions in mitotic activity of 14, 17, 48 and 81% at concentrations of 6.25, 12.5, 25.0 and 40.0  $\mu\text{g/ml}$  respectively. During scoring, a reduction in the quantity of cells present was noted on slides from cultures treated at 40.0  $\mu\text{g/ml}$ . At the 48 hour sampling time, no toxicity was seen in cultures treated at 6.25, 12.5 or 25.0  $\mu\text{g/ml}$ : reductions in mitotic activity of 40 and 81% were seen at 35.0 and 45.0  $\mu\text{g/ml}$ . During scoring, a reduction in the quantity of cells present was noted on slides from cultures treated at 45.0  $\mu\text{g/ml}$ .

Main cytogenetic test - group totals

Compound (ug/ml)	Numbers of cells scored	Mean mitotic index	Cells with aberrations			Cells with aberrations other than gaps		
			Total	Indiv. values	+ Mean %	Total	Indiv. values	+ Mean %
<b>24 hour sampling time</b>								
<u>Non-activated cultures</u>								
DMSO (-)	200	8.6	6	2,4	3.0	4	1,3	2.0
SR 33589 B (1.25)	200	8.7	7	3,4	3.5	3	1,2	1.5
SR 33589 B (2.5)	200	6.1	5	2,3	2.5	2	1,1	1.0
SR 33589 B (5.0)	200	3.0	14	6,8	7.0	10	5,5	5.0
Chlorambucil (2.0)	200	9.2	64	31,33	32.0	55	26,29	27.5
<u>Activated cultures</u>								
DMSO (-)	200	9.3	7	3,4	3.5	4	1,3	2.0
SR 33589 B (6.25)	200	8.0	9	4,5	4.5	6	3,3	3.0
SR 33589 B (12.5)	200	7.7	11	4,7	5.5	5	2,3	2.5
SR 33589 B (25.0)	200	4.8	14	5,9	7.0	7	2,5	3.5
Cyclophosphamide (6.0)	200	9.5	68	33,35	34.0	53	26,27	26.5
<b>48 hour sampling time</b>								
<u>Non-activated cultures</u>								
DMSO (-)	200	8.8	5	2,3	2.5	4	1,3	2.0
SR 33589 B (1.25)	200	7.8	7	3,4	3.5	4	1,3	2.0
SR 33589 B (2.5)	200	6.8	5	1,4	2.5	2	0,2	1.0
SR 33589 B (5.0)	163	1.6	6	3,4,8	3.7	4	1,6,3	2.5
Chlorambucil (2.0)	200	10.1	26	12,14	13.0	18	9,9	9.0
<u>Activated cultures</u>								
DMSO (-)	200	7.8	4	2,2	2.0	2	1,1	1.0
SR 33589 B (12.5)	200	9.2	3	0,3	1.5	0	0,0	0.0
SR 33589 B (25.0)	200	9.3	10	3,7	5.0	5	2,3	2.5
SR 33589 B (35.0)	200	4.7	10	4,6	5.0	6	1,5	3.0
Cyclophosphamide (6.0)	200	10.8	25	11,14	12.5	22	11,11	11.0

$$+ \text{ Mean \%} = \frac{\text{No. aberrant metaphases}}{\text{Total cells scored}} \times 100$$

A slight increase in aberrations was seen both ± S9. The responses from the positive control were not always robust, as shown at the 48 hour time point.

Main cytogenetic test - group totals

Compound (ug/ml)	Numbers of cells scored	Mean mitotic index	Cells with aberrations			Cells with aberrations other than gaps		
			Total	Indiv. values %	+ Mean %	Total	Indiv. values %	+ Mean %
<b>24 hour sampling time</b>								
<u>Non-activated cultures</u>								
DMSO (-)	200	8.6	6	2,4	3.0	4	1,3	2.0
SR 33589 B (1.25)	200	8.7	7	3,4	3.5	3	1,2	1.5
SR 33589 B (2.5)	200	6.1	5	2,3	2.5	2	1,1	1.0
SR 33589 B (5.0)	200	3.0	14	6,8	7.0	10	5,5	5.0
Chlorambucil (2.0)	200	9.2	64	31,33	32.0	55	26,29	27.5
<u>Activated cultures</u>								
DMSO (-)	200	9.3	7	3,4	3.5	4	1,3	2.0
SR 33589 B (6.25)	200	8.0	9	4,5	4.5	6	3,3	3.0
SR 33589 B (12.5)	200	7.7	11	4,7	5.5	5	2,3	2.5
SR 33589 B (25.0)	200	4.8	14	5,9	7.0	7	2,5	3.5
Cyclophosphamide (6.0)	200	9.5	68	33,35	34.0	53	26,27	26.5
<b>48 hour sampling time</b>								
<u>Non-activated cultures</u>								
DMSO (-)	200	8.8	5	2,3	2.5	4	1,3	2.0
SR 33589 B (1.25)	200	7.8	7	3,4	3.5	4	1,3	2.0
SR 33589 B (2.5)	200	6.8	5	1,4	2.5	2	0,2	1.0
SR 33589 B (5.0)	163	1.6	6	3,4,8	3.7	4	1,6,3	2.5
Chlorambucil (2.0)	200	10.1	26	12,14	13.0	18	9,9	9.0
<u>Activated cultures</u>								
DMSO (-)	200	7.8	4	2,2	2.0	2	1,1	1.0
SR 33589 B (12.5)	200	9.2	3	0,3	1.5	0	0,0	0.0
SR 33589 B (25.0)	200	9.3	10	3,7	5.0	5	2,3	2.5
SR 33589 B (35.0)	200	4.7	10	4,6	5.0	6	1,5	3.0
Cyclophosphamide (6.0)	200	10.8	25	11,14	12.5	22	11,11	11.0

+ Mean % =  $\frac{\text{No. aberrant metaphases}}{\text{Total cells scored}} \times 100$

TABLE 5

Main cytogenetic test - group totals of specific aberrations

Compound (ug/ml)	CTG	CSG	CTB	CTF	CSF	CTE	CSE	P	End	Cells with >1 aberration	
										+g	-g
<b>24 hour sampling time</b>											
<u>Non-activated cultures</u>											
DMSO (-)	2	0	2	1	2	0	0	0	0	1	1
SR 33589 B (1.25)	4	0	2	0	1	0	0	2	0	0	0
SR 33589 B (2.5)	2	1	2	0	0	0	0	0	0	0	0
SR 33589 B (5.0)	4	1	8	1	3	0	0	2	0	2	2
Chlorambucil (2.0)	14	2	44	0	10	6	0	0	0	12	5
<u>Activated cultures</u>											
DMSO (-)	3	0	3	0	1	0	0	2	0	0	0
SR 33589 B (6.25)	3	0	6	0	0	0	0	3	0	0	0
SR 33589 B (12.5)	6	0	4	0	1	0	0	2	0	0	0
SR 33589 B (25.0)	8	0	3	0	4	0	0	2	0	1	0
Cyclophosphamide (6.0)	19	2	45	2	8	10	0	2	0	12	8
<b>48 hour sampling time</b>											
<u>Non-activated cultures</u>											
DMSO (-)	1	0	7	0	0	0	0	3	0	1	1
SR 33589 B (1.25)	3	0	3	0	1	0	0	0	0	0	0
SR 33589 B (2.5)	3	0	0	0	2	0	0	4	0	0	0
SR 33589 B (5.0)	3	0	2	1	1	0	0	3	0	1	0
Chlorambucil (2.0)	9	0	6	1	13	0	0	0	1	3	2
<u>Activated cultures</u>											
DMSO (-)	3	0	1	1	0	0	0	2	0	1	0
SR 33589 B (12.5)	3	0	0	0	0	0	0	3	0	0	0
SR 33589 B (25.0)	5	0	3	0	2	0	0	6	0	0	0
SR 33589 B (35.0)	3	1	4	2	0	0	0	7	0	0	0
Cyclophosphamide (6.0)	3	0	12	1	9	1	1	3	0	2	2

CTG Chromatid gap      CSF Chromosome fragment      End Endoreduplicated cell  
 CSG Chromosome gap      CTE Chromatid exchange      +g All aberration types included  
 CTB Chromatid break      CSE Chromosome exchange      -g All aberration types except gaps included  
 CTF Chromatid fragment      P Polyploid cell

**Main cytogenetic test - statistical analysis of the frequency of chromosomal aberrations - comparison between treated and control groups**

Compound	----- SR 33589 B -----							CBC	CP	
Concentration (ug/ml)	1.25	2.5	5.0	6.25	12.5	25.0	35.0	2.0	6.0	
S-9 Mix	-	-	-	+	+	+	+	-	+	
<b>24 hour sampling time:</b>										
Including gaps (single-sided test)	p-value	0.500	0.729	0.053	0.400	0.235	0.089	-	<0.001	<0.001
	significance	NS	NS	NS	NS	NS	NS	-	***	***
Excluding gaps (single-sided test)	p-value	0.776	0.892	0.086	0.375	0.500	0.272	-	<0.001	<0.001
	significance	NS	NS	NS	NS	NS	NS	-	***	***
<b>48 hour sampling time:</b>										
Including gaps (single-sided test)	p-value	0.386	0.625	0.363	-	0.776	0.086	0.086	<0.001	<0.001
	significance	NS	NS	NS	-	NS	NS	NS	***	***
Excluding gaps (single-sided test)	p-value	0.638	0.892	0.521	-	1.000	0.225	0.142	0.002	<0.001
	significance	NS	NS	NS	-	NS	NS	NS	**	***
CBC Chlorambucil	NS Not significant, p > 0.05									
CP Cyclophosphamide	** Highly significant, 0.01 > p > 0.001									
	*** Very highly significant, p < 0.001									

**Study title:** Micronucleus test in vivo genotoxicity study by the oral route in the mouse.

**Key findings:** There was no increase in micronucleated nuclei in the data as presented.

**Study no.:** Mut0046

**Conducting laboratory and location:** Sanofi Recherche, Montpellier Cedex, France

**Date of study initiation:** July 7, 1993

**GLP compliance:** statement included

**QA reports:** yes (x) no ( )

**Drug, lot #, and % purity:** SR33589B batch 92.011-11, purity, suspension in 0.6% methylcellulose

Positive control of cyclophosphamide given intraperitoneally in aqueous solution

## Methods

Male and females mice OF1 (UIOPS) were used. The treatment groups are summarized below:

### Summary of study design

# of animals per group	# of the first animal	compound	route	Dose mg/kg
10 males 10 females	01 11	0.6% methylcellulose	po	0
5 males 5 females	21 26	endoxan	ip	50
12 males 12 females	31 43	SR33589B	po	2000

Animals were euthanized 24 and 48 hours after dosing. One slide per animal was scored. PCEs and NCEs were counted until 2000 PCEs were analyzed.

## Results

No clinical signs, toxicity or mortality were reported for any of the animals treated with SR33589B.

### 2.6.6.5 Carcinogenicity

The Exec CAC minutes of the dose selection process are attached as Appendix A. The review of the mouse and rat carcinogenicity studies is included as Appendix B. The Exec CAC minutes of the assessment of the results are attached as Appendix C.

### 2.6.6.6 Reproductive and developmental toxicology

#### *Fertility and early embryonic development*

**Study title:** Preliminary Segment I study in the rat

**Key study findings:** Estrous cycle irregularity or acyclicity was seen in the drug-treated animals. There were decreased numbers of corpora lutea and subsequently decreased implantations and live fetuses. Males were apparently subject only to macroscopic observations. No data was found regarding sperm count, motility or morphology.

**Study no.:** FER250

**Conducting laboratory and location:** Huntingdon Life Sciences, Suffolk, England

**Date of study initiation:** January 17, 1996

**GLP compliance:** statement included

**QA reports:** yes ( ) no ( )

**Drug, lot #, and % purity:** SR33589B batch 5SNP505 in 0.6% methylcellulose

**Methods** Doses of 0, 10, 30 and 100 mg/kg/day were given by oral gavage to Sprague-Dawley rats, 6/sex/group. Males received the drug from 15 days before pairing to Day 36 of treatment. Females received the drug from 15 days before pairing to day 7 after mating. Males were euthanized after the females on day 36 of treatment (Day 14 of gestation).

**Results**

Both sexes showed dose related salivation at doses  $\geq 30$  mg/kg/day.

Females at 100 mg/kg showed hairloss on one or more regions of the body.

The HD males gained ~10% less than the control group.

Body weight: males (grams)

Dose mg/kg/day	Week 0	Week 5(final)	
0	334± 10	480±45	146(44%)
10	333± 6	469±18	136(41%)
30	329± 8	468 ±18	139(42%)
100	334± 7	442±22	108(32%)

HD females gained less weight than did the control groups in both the pre-mating period and during gestation.

Body weight: females (grams)

Dose mg/kg/day	Week 0	Week 2	Day of gestation			from GD0
			Treatment period GD0	GD7	GD14	
0	228±7	281±13	287±21	329±22	374±26	87(30%)
from base		53 (23%)				
10	227±5	281±7	293±20	335±16	382±16	89(30%)
30	230±9	280±12	285±13	323±17	374±25	89(31%)
100	230±7	262±14	273±11	297±19	345±21	72(26%)
		32(14%)				

Food consumption was decreased in the HD groups of both sexes in the first 2 weeks of the study. Thereafter food consumption was similar across groups.

The overt effects are summarized below. Five out of 6 of the HD females conceived. The mean number of corpora lutea were decreased at the HD and subsequently the number of implantations and liver fetuses. There was some estrous cycle irregularity in the LD and MD groups.

Summary of main reproduction parameters : total number of litters or mean per litter±SD

	Dose mg/kg/day			
	0	10	30	100
Corpora lutea	18.2±2.0	19.2±2.1	19.3±1.9	14.8±2.6
Implantations	16.3±2.8	16.8±1.7	18.3±2.0	12.0±5.5
Pre-implantation loss %	10.1	12.2	5.2	18.9
Live fetuses	15.2±2.8	15.3±1.8	17.0±1.3	11.2±5.1
Dead fetuses	0	0	0	0
Early resorptions	1.17±1.08	1.50±1.22	1.33±1.15	1.00±1.00
Late resorptions	0	0	0	0
Post-implantation loss%	7.1	8.9	7.3	6.7
Estrous Cycles Arithmetic number (percent)				
Regular 4 or 5 day cycle	6(100)	4(67)	5(83)	6(100)
Irregular cycle <sup>a</sup>	0	1(17)	1(17)	0
Acyclic <sup>b</sup>	0	1(17)	0	0

<sup>a</sup> At least one cycle of two, 3, or six-10 days

<sup>b</sup> At least 10 days without estrous

**Study title:** Study of effects on fertility and early embryonic development in CD rat by oral gavage administration FER0297

**This study was previously reviewed (Amendment 062) by this reviewer. A summary will be provided here.**

Doses of 10, 30 and 100 mg/kg/day were used. The same study design was used as in the preliminary study. The highest dose again produced some degree of maternal toxicity as the HD females showed body weight gain 8% lower than that of the control group. Water consumption was increased in the MD and HD males by up to 16% compared to controls. Prior to mating water consumption was increased in all the drug-treated female groups by up to 45%. During gestation, HD females consumed up to 20% more water than the controls.

Other significant findings included the tendency to decreased numbers of regular estrus cycles (significant at the MD and HD levels), consistent with the preliminary study. There was also a non-dose dependent tendency to acyclic females in the drug-treated groups. Consistent with the preliminary study was a decreased number of corpora lutea, implantations and live fetuses. Effects such as decreases in live fetuses, increases in early resorptions and increases in post implantation losses were confined to the HD group and may be associated with maternal toxicity.

Pre-implantation loss was increased somewhat. Sperm count and motility as well as weight of testes and epididymides were provided. Sperm count estimates were taken from the vaginal smears at mating. Another table was entitled "Sperm analysis" but it is not clear that this was data was obtained through separate analysis.

## Summary of reproduction parameters

	Dose mg/kg			
	0	10	30	100
Corpora lutea implantations	17.1±2.3	17.1±1.8	17.8±2.0	15.7±4.1
Pre-implantation loss %	16.0±3.1	16.7±1.7	17.0±2.2	13.5±5.3 <sup>a</sup>
Live fetuses	7.6	3.2	4.8	14.6
Early resorptions	15.5±2.8	15.3±2.6	16.1±2.4	11.7±5.1 <sup>a</sup>
Post-implantation loss%	0.55±0.74	1.41±1.19	0.77±0.88	1.80±1.34 <sup>a</sup>
4 day estrous cycle	20(91)	18(82)	10(45) <sup>b</sup>	11(50) <sup>b</sup>
4/5 day estrous cycle	1(5)	2(9)	4(18)	9(41) <sup>b</sup>
5 day estrous cycle	0	0	4(18)	0
Irregular	1(5)	0	0	1(5)
Acyclic	0	2(9)	4(18)	1(5)

at least one cycle of 2,3 or 6-10 days

at least 10 days without estrous

## Pre-coital interval summary : mean (%)

Dose mg/kg # animals	Pre-coital interval (days)				
	1-4	5-8	9-12	13-16	17-21
0 n=22	21(95)	1(5)	0	0	0
10 n=22	22(100)	0	0	0	0
30 n=22	17 (77)	2(9)	2(9)	0	1(5)
100 n=22	15 (68)	7(32)	0	0	0

The female NOAEL was 10 mg/kg/day based upon the body weight effects, decrease in regular cycles and alterations in pre-coital interval.

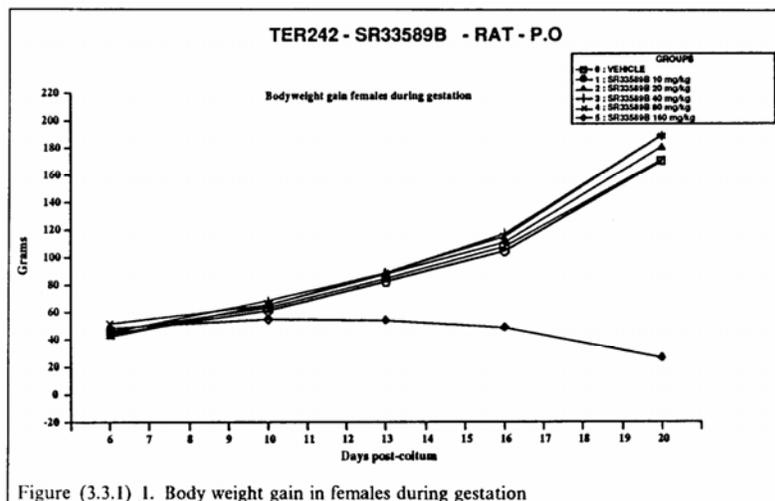
### ***Embryofetal development***

The embryofetal studies (Segment II) were previously reviewed and will be summarized here.

#### **TER0242 Preliminary teratology study in the rat**

Doses used: 0, 10, 20, 40, 80, 160 mg/kg/day in 0.6% methylcellulose from GD6-GD15

Mortality was seen at the HD (GD14 and GD15). There was a slight decrease in maternal body weight gain at 80 mg/kg from GD6-GD10 and markedly decreased body weight at 160 mg/kg from GD10 onwards. One case of vaginal bleeding was seen between days 15 and 17 in the HD group.



The premature decedents in the HD group showed small hemorrhagic thymuses, distended hearts, lungs with grey areas, enlarged hemorrhagic adrenals, meteorism and liquid contents in the GI tract.

The HD group showed slight increases in pre-implantation losses, resorptions and post-implantation loss. Mean fetal weight and placental weight were significantly decreased in this group also.

Fetal malformations were increased at 80 mg/kg/day and at 160 mg/kg/day. In the HD group, 33 fetuses (89%) had at least 1 external abnormality compared to 1 control fetus (1%) and 0 in the 10, 20 40 and 80 mg/kg groups. Several fetuses presented with internal malformations related to sexual differentiation. To quote the sponsor:

In the control group, 1 fetus out of 92 observed presented a caudal malformation: Anuria, anal imperforation, undifferentiated sexual characteristics (classified as male Fetus).

At 160 mg/kg/d (group 5), 33 of 37 fetuses presented malformations, multiple and often associated together ( in 2 of the 3 litters). The main areas concerned were:

- the face (brachygnathia and/or cleft palate)
- the limbs (anterior and/or posterior clubfeet)
- the fingers (ectrodactylia and/or syndactylia)
- the tail (deviations, partial reduction)
- the anogenital area (undiscernible anogenital space, suspected anogenital fistula, prominent genital papilla). [page 25]

At 160 mg/kg 17 of 18 fetuses examined for skeletal formation showed at least one skeletal malformation: vertebrae, limbs, thoracic cage. The sponsor also noted that fetuses with external malformations also presented with generalized delayed ossification. The internal malformations noted in this group were all related to sexual differentiation and were reported with one particular litter. Three male fetuses showed ectopic testes (gonads in lumbar area). Three female

fetuses had uterine horns around the kidneys, ectopic ovaries (anterior to the kidneys) and 1 fetus had no sexual differentiation and gonads anterior to the kidneys.

At 80 mg/kg malformations were reported for 15 of the 48 fetuses examined. These were primarily of the skeletal system: thoracic cage, limbs and vertebrae. It was also reported that there was an increase in delayed ossification of cervical and thoracic centers and of sternbrae as well. An increased number of asymmetric sternbrae was reported.

The lower doses ( $\leq 40$  mg/kg) did not have malformations. Delayed ossification was observed in cervical and thoracic centers.

Teratologic effects were seen at a maternally toxic dose (160 mg/kg/day) and also with no maternal toxicity(80mg/kg/day).

### TER0244 Teratology study in the rat

This study was previously reviewed and is summarized here.

Doses of 0, 10, 30 and 100 mg/kg/day were given in 0.6% methylcellulose from GD6-GD15.

There was a decreased rate of weight gain in the HD group.

Early and late resorptions were increased at the HD as were post-implantation losses.

	Dosage mg/kg/day				Historical control data	
	0	10	30	100	16256 feti	56 studies
# of fetuses (litters examined)	307(22)	324(22)	315(22)	283(22)		
# of male:female fetuses	159:148	149:175	147:168	127:156		
Observations: incidence (# of litters)						
Small fetus <2.80g	1.0(3)	2.5(3)	1.6(3)	33.2(14)	1.45	0.0-4.9
Large fetus <4.10 g	10.1(9)	5.9(9)	10.8(14)	2.5(3)	8.27	0.0-20.8
Shiny fetus	0.3(1)	0.3(1)	0.6(2)	1.8(3)	0.33	0.0-2.7
Mottled fetus syndrome*	0.0(0)	1.5(1)	0.0(0)	0.00(0)	#	#
Severe encephalocele	0.0(0)	0.0(0)	0.0(0)	1.8(2)	0.01	0.0-0.7
Domed head	0.0(0)	0.0(0)	0.0(0)	2.5(2)	0.10	0.0-0.7
Unilateral anophthalmia	0.0(0)	0.3(1)	0.0(0)	1.4(3)	0.01	0.0-0.5
Bilateral anophthalmia	0.0(0)	0.0(0)	0.0(0)	3.2(3)	0.01	0.0-0.4
Unilateral microphthalmia	0.0(0)	0.3(1)	0.0(0)	0.7(2)	0.01	0.0-0.5
Bilateral microphthalmia	0.0(0)	0.0(0)	0.0(0)	1.1(2)	#	#

# no record in background control data

	Dosage mg/kg/day				Historical control data	
	0	10	30	100	16256 feti	56 studies
# of fetuses (litters examined)	307(22)	324(22)	315(22)	283(22)		
# of male:female fetuses	159:148	149:175	147:168	127:156		
Observations: incidence (# of litters)						
Pointed/upturned snout	0.0(0)	0.0(0)	0.0(0)	3.2(4)	0.06	0.0-2.2
length lower jaw	0.0(0)	0.0(0)	0.0(0)	5.7(5)	#	#
Protruding tongue	0.0(0)	0.0(0)	0.0(0)	0.4(1)	0.02	0.0-1.3
Cleft palate	0.0(0)	0.0(0)	0.0(0)	29.3(10)	#	#
Punctuate dark area/ prom-inent blood vessel on palate	0.3(1)	0.0(0)	0.0(0)	0.4(1)	0.01	0.0-0.3
Edema on body	0.0(0)	0.0(0)	0.0(0)	7.4(6)	0.02	0.0-0.5
Hemorrhage on body	0.0(0)	0.0(0)	0.6(2)	0.7(1)	0.01	0.0-0.3
Umbilical hernia	0.0(0)	0.0(0)	0.0(0)	0.7(1)	0.02	0.0-0.3
Inward curvature of spine at thorax	0.0(0)	0.0(0)	0.0(0)	1.8(1)	#	#

# no record in background data

While some of the anomalies listed fall within the range of historical values, all of the defects are found in the same dose group, not distributed amongst all the groups.

	Dosage mg/kg/day				Historical control data	
	0	10	30	100	16256 feti	56 studies
# of fetuses (litters examined)	307(22)	324(22)	315(22)	283(22)		
# of male:female fetuses	159:148	149:175	147:168	127:156		
Observations: incidence (# of litters)						
Unilateral forelimb flexure	0.0(0)	0.0(0)	0.0(0)	1.1(3)	0.02	0.0-3.5
Bilateral forelimb flexure	0.0(0)	0.0(0)	0.0(0)	0.7(2)	0.20	0.0-3.5
forelimbs in length/misshapen	0.0(0)	0.0(0)	0.0(0)	1.4(3)	0.01	0.0-3.3
Forepaws misshapen +/- or in size	0.0(0)	0.0(0)	0.0(0)	2.5(2)	#	#
≥2 digits fused	0.0(0)	0.0(0)	0.0(0)	6.7(8)	#	#
Agenesis ≥1 digit	0.0(0)	0.0(0)	0.0(0)	23.7(10)	#	#
digital size	0.0(0)	0.0(0)	0.0(0)	0.4(1)	#	#
Hindlimbs length/misshapen	0.0(0)	0.0(0)	0.0(0)	4.6(5)	#	#
Hindlimbs malrotated	0.0(0)	0.0(0)	0.0(0)	8.5(7)	0.02	0.0-0.6
Tail in length/kinked/tip curled/rudimentary	0.0(0)	0.0(0)	0.0(0)	37.8(12)	0.01	0.0-0.7

# no record in background data

The sponsor’s summary of the data indicated that increased fetal effects were reported only in the HD group, associated with maternal toxicity. As noted in the original review:

...the rats showed maternal toxicity at the HD-100 tested and marked adverse effects on embryo-fetal development. Compared to control, embryo-fetal toxicity induced by the 100 mg/kg/day consisted in statistically significant increase in post-implantation losses (p<0.05), reduced fetal/placental

weights ( $p < 0.001$  and  $p < 0.01$  respectively), and increased [sic] in the number of rat litters with fetuses showing external, visceral and skeletal malformations (e.g., cranioschisis, cleft palate, incomplete evagination of pineal body, brachygnathia, partially fused carotid arteries, truncus arteriosus, abnormal lobation of the liver, partially duplicated inferior vena cava, brachydactyly, etc.) It should be noted that the structurally related drug amiodarone at 200 mg/kg/day (18X its the [sic] maximum recommended dose) administered to rats during organogenesis was shown to be embryotoxic. This marketed drug is labeled as Pregnancy category D (“... can cause fetal harm when administered to a pregnant woman...”).

It was noted in the report for dpn0295, “Study of the effects of SR33589B on pre- and post-natal development” that

In a preliminary teratology study conducted in the rat [2], females were administered dosages of 0, 10, 20, 40, 80 or 160 mg/kg/d from GD6-15. At a dosage of 160 mg/kg/d, maternal toxicity occurred, including death and decreases in body weight and food consumption. In addition, increases in pre-implantation loss, resorptions, and post-implantation loss were accompanied by decreases in mean fetal body weight, placental weight, and litter size. Upon examination of fetuses, numerous external, skeletal and visceral malformations were observed. At a dosage of 80 mg/kg/d, 30% of the fetuses examined had skeletal malformations, whereas no external malformations were observed. No visceral examinations were performed for the 80 mg/kg/d dosage group.

In the definitive teratology study [3], female rats received dosages of 0, 10, 30 or 100 mg/kg/d from GD6-15. At 100 mg/kg/d, females exhibited decreases in body weight gain and food consumption during the dosing period. A moderate increase in post-implantation loss was noted, as were decreases in litter size, fetal weight, and placental weight. In addition, 198 of 221 fetuses exhibited external, skeletal and/or visceral major malformations. No maternal or fetal effects were seen at dosages of 10 or 30 mg/kg/d.

As terata were seen at a dose of 80 mg/kg with no maternal toxicity, a dose between 30 and 100 might have been useful to define better the NOAEL.

#### **DD0518 13-day oral dose range-finding study in female rabbits**

The study is summarized here.

Doses used were 0, 50, 100, 200 mg/kg/day as a suspension in 0.6% methylcellulose given from GD1 to GD13. Rabbits were euthanized GD14. There were no treatment related changes reported for clinical signs, mortality, body weight or food intake. No lesions were reported in the macroscopic examination. Minimal detail was included in this 29 page report.

#### **TER0241 Preliminary teratology study in the rabbit**

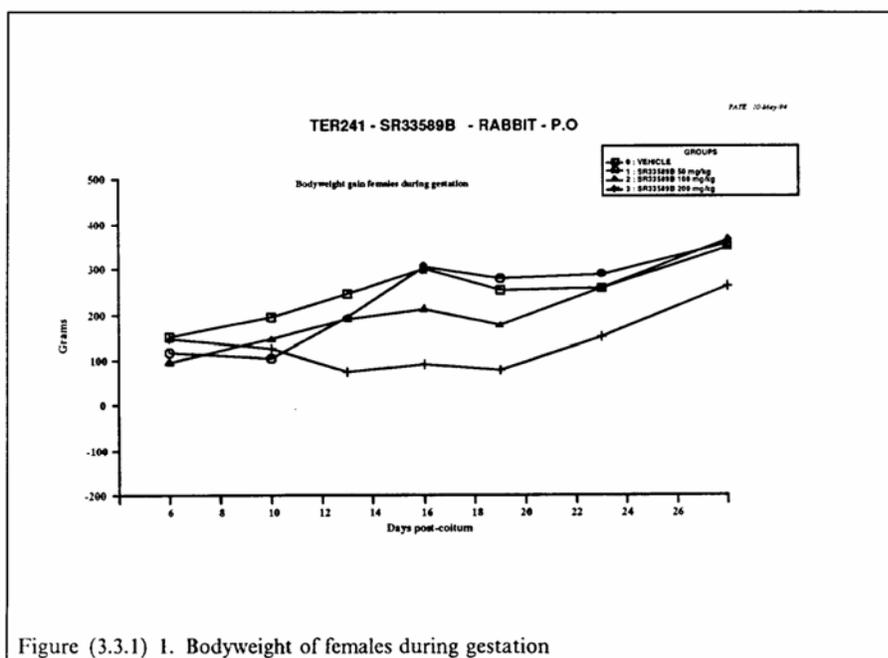
This study was previously reviewed and is summarized here.

Doses of 0, 50, 100, 200 and 400 mg/kg/day were given by oral gavage in 0.6% methylcellulose from GD6 to GD18. Rabbits were euthanized GD28.

Maternal toxicity was seen at the HD. The bodyweight changes for this group were not analyzed. Unscheduled mortality was reported:

- 0 -
- 50 1 (day 15), maybe accidental
- 100 1(day 23) subacute bronchopneumonia
- 200 1(day 9) subacute bronchopneumonia
- 400 6 (between day 10 and day 12)

There was a body weight effect at the next lower dose of 200 mg/kg.



Only skeletal and external examinations were performed. The fetal examinations showed no external or skeletal malformations in the live fetuses. It was reported that no malformations were apparent in the live fetuses. It is not clear from the report how many fetuses were available to make this determination. One dead fetus showed multiple malformations that included abdominal celosomia, absence of caudal formation, fusion of hindlimbs (monopodia) with malformation of digits.

The macroscopic observations on the premature decedent dams included

- 400 mg/kg: intestinal meteorism, pale heart
- ≥200 mg/kg lung congestion and hemorrhagic thymus
- 100 mg/kg vaginal bleeding, pale heart and liver, intestinal meteroism

The study accomplished its goal of determining the HD for the definitive SegII rabbit study.

**TER0243 Study of effects on embryo-fetal development in the rabbit.**

This study was previously reviewed and is summarized here.

Doses of 0, 20, 60 and 200 mg/kg was given as an oral gavage in 0.6% methocel from GD6 to GD18. Dams were euthanized GD29. Unscheduled mortality was seen in the HD group: 1 HD f was euthanized in extremis and 4 were found dead. Nine out of 18 females in the hd group aborted.

Body weight effects were seen in the MD and HD groups. Food consumption was significantly decreased by 30-73% ( $p < 0.001$ ) in the HD group during the treatment period.

A dose related decrease in live fetuses was seen. Post-implantation loss was seen only in the HD group. Pre and post-implantation losses were increased with drug treatment. A non-dose related increase in malformations was reported.

Summary of main reproduction parameters (mean per litter  $\pm$ SD)

	Dose mg/kg			
	0	20	60	200
Euthanized or found dead before day 29	0	0	1	6 <sup>a</sup>
Aborted females	2	0	0	9
Evaluated pregnant females w/ fetuses/mated	18/22	19/22	18/22	5/22
Copora lutea	12.9 $\pm$ 2.1	14.1 $\pm$ 2.7	12.0 $\pm$ 2.5	12.8 $\pm$ 2.6
implantations	11.7 $\pm$ 2.8	12.1 $\pm$ 3.2	10.1 $\pm$ 2.7	9.2 $\pm$ 1.9
Preimplantation loss %	9.8	14.9	16.6	28.1
Live fetuses	9.4 $\pm$ 2.9	9.4 $\pm$ 3.2	8.4 $\pm$ 3.2	6.0 <sup>b</sup> $\pm$ 3.5
Post-implantation loss %	19.9	21.8 <sup>c</sup>	16.6	34.8
Weight of fetuses (g)	37.4 $\pm$ 2.5	38.6 $\pm$ 1.9	40.2 $\pm$ 1.9	40.5 $\pm$ 2.5
Weight of placenta (g)	4.8 $\pm$ 0.3	4.9 $\pm$ 0.2	5.3 $\pm$ 0.3	5.3 $\pm$ 0.3
Main fetal abnormalities				
Multiple malformations of head		3 <sup>d</sup>		
Anomalous ribcage + vertebral column		1 <sup>e</sup>		1
Anomalous thoracic vertebrae		1 <sup>e</sup>		
Brachygnathia and cleft palate			1	

<sup>a</sup> including 2 not-pregnant females; <sup>b</sup> significant compared to the controls  $p < 0.05$ ; <sup>c</sup> includes one dead fetus

<sup>d</sup> separate litters; <sup>e</sup> same litter

Two fetuses, both in the 20 mg/kg group but from different litters were grossly abnormal. One fetus showed a proboscis, “reduced” head, both ears pointing forwards, cyclopia, agenesis of the lower jaw and mouth and enlarged and misshapen larynx. The other showed large and misshapen larynx, enlargement and central displacement of both eyes with partially open eyelids, domed head, enlarged snout with agenesis of nares and agenesis of lower jaw with no mouth. The sponsor felt that these animals were affected by “Froxfield Head Syndrome.”

Both the 20 and 200 mg/kg groups showed an incidence of incomplete ossification of the hyoid body that was elevated compared to current and historical controls. An increased incidence of rib anomalies was also noted in the drug treated groups.

Observations : % fetal incidence (# of litters)	Dose mg/kg				Historical Control data	
	0	20	60	200	1043 feti	11 studies
Incomplete ossification of hyoid body	34.5(14)	42.9 (15)	31.0 (14)	45.0(3)	22.15	6.8-41.9
Asymmetric pelvis	4.7(6)	6.7(9)	6.0 (6)	6.7(1)	5.26	3.6-7.9

There are no clear fetal effects in this study. It is however somewhat troubling that a dose that caused minimal toxicity in the preliminary study caused substantial maternal toxicity in the definitive study.

A maternal NOAEL was 20 mg/kg while the fetal NOAEL is 60 mg/kg/day based upon post-implantation losses. The sponsor felt that 200 mg/kg was a fetal NOAEL but noted that only 5 litters were available to evaluate at this dose.

### *Prenatal and postnatal development*

#### **DPN0295 Study of the effects of SR33589B on pre- and post-natal development (including maternal function) in the rat by oral gavage**

This study was previously reviewed and is summarized here.

Doses of 0, 10, 30 and 50 mg/kg/day were given by oral gavage as suspensions in 0.6% methylcellulose. The F0 females were dosed once daily from GD6 to lactation day 20 or day prior to necropsy, whichever was later.

Days of scheduled necropsies:

F1 generation(unselected): PPD4: culled to 5/sex/litter where possible

PPD21-23: males and females not selected for behavioral or

Reproductive assessment reduced to 2/sex/litter

Where possible.

F1 generation(selected for : PPD67-78 after completion of auditory startle tests

Behavioral assessment)

F1 generation(selected for: Females GD20(~20 days after last day of cohabitation)

Reproductive assessment) Males PPD118-127 after necropsy of F1 females

## Summary of findings in the F0 generation

Observation	Dose mg/kg			
	0	10	30	50
Inseminated females	25	25	25	25
Females delivered (# of litters)	25	24	24	23
Viability index PPD4 (pre-culling)	376/380 (99.1%)	369/378 (97.6%)	388/392 (99.1%)	347/370 (93.8%)
Weight of pups (g) PPD1	6.3±0.1	6.2±0.1	6.1±0.1	5.9±0.1
Weight of pups (g) PPD21	50.9±0.8	48.2±1.1	49.8±1.0	47.6±1.5
F1 generation				
Inseminated females	25	24	23	22
Weight of fetuses (g)	3.26±0.10	3.21±0.05	3.12±0.07	3.12±0.08
Weight of placenta (g)	0.59±0.01	0.55±0.01	0.56±0.01	0.51±0.01*
Observations in F1 pups				
Any variations in the eyes: corneal opacity Litter %	0	0	0	1 pup in 1 litter 4%
Malrotated hindlimbs Litter %	0	1 pup in 1 litter 4%	0	0
Small bulgey eyes Litter %	0	0	0	2 pups in 1 litter 4%

\*significantly different from control at p&lt;0.05

Below is a summary of the times at which the different groups were assessed.

F0 females	
GD0	Day of insemination
GD6-D20 of lactation ( or day prior to necropsy)	Tx of F0 females
D21 of lactation ( or nearest workday thereafter)	Scheduled necropsy of F0 females that delivered
25 days after presumed GD0	Scheduled necropsy of non-pregnant F0 female
F1 males and Females	
Selected and unselected pups (until weaning)	
PPD1	Day of birth
PPD2	Static righting reflex evaluation begun
PPD4	Litters culled to 5/sex/litter where possible
PPD12	Eye opening evaluation begun
PPD21-23	Weaning. Litter size to 2/sex where possible Scheduled necropsy of unselected pups
Pups selected for behavioral assessment (series 3 and 4)	
PPD28	Vaginal opening evaluation begun
PPD28 ( $\pm 5$ days), PPD35( $\pm 5$ days)	Passive avoidance test
PPD35	Balanopreputial separation evaluation begun
PPD60 ( $\pm 5$ days)	Auditory startle test
PPD67-78	Scheduled necropsy of males and females
Pups selected for reproductive assessment (series 5 and 6)	
PPD28	Vaginal opening evaluation begun
PPD35	Balanopreputial separation evaluation begun
PPD65( $\pm 5$ days)	Open field motor activity test
$\geq$ PPD71	Estrus determination
$\geq$ PPD85	Cohabitation
GD0	Day of insemination
GD20	Scheduled necropsy of inseminated females
~20 days after cohabitation	Scheduled necropsy of non-inseminated females
PPD118-127	Scheduled necropsy of males

### F1 reproductive performance

There was no apparent effect upon pre-mating estrous cycle and time to insemination data for F1 females, nor upon the course of pregnancy data.

There was a slight increase in resorptions for the mated F1 females

Observations in F1 females Mean±SE (range)

	Dose mg/kg			
	0	10	30	50
resorptions	0.6±0.1 (0-2)	0.8±0.2(0-4)	0.9±0.2(0-4)	1.0±0.3(0-5)
Post-implantation loss%	3.9±0.9 (0-13)	6.1±2.0 (0-33)	5.4±1.5 (0-25)	5.9±1.8 (0-28)
Resorptions %	3.9±0.9 (0-13)	6.1±2.0 (0-33)	5.4±1.5 (0-25)	5.9±1.8 (0-28)

While the F1 offspring of the HD females weighed more than the control group, the ovarian weight was lower.

Body and organ weight data for F1 females (mean±se)

	Dose mg/kg			
	0	10	30	50
Body weight (g)	445.9±8.3	431.6±8.9	441.9±9.4	454.9±8.9
Ovary weight (g)	0.228±0.008	0.204±0.009	0.212±0.009	0.206±0.009

F2 pups weighed (non-significantly) less than the control pups.

Body weight of F2 pups (mean±se)

	Dose mg/kg			
	0	10	30	50
Average male (range)	3.37±0.10 (2.6-5.2)	3.30±0.05 (2.9-3.6)	3.22±0.08 (2.5-3.6)	3.21±0.08 (2.6-3.9)
Average female (range)	3.15±0.10 (2.4-4.9)	3.11±0.05 (2.7-3.5)	3.04±0.07 (2.5-3.4)	3.04±0.08 (2.3-3.6)
Average female placental weight (g)	0.58±0.01	0.55±0.02	0.56±0.02	0.50±0.01*
Average male placental weight (g)	0.60±0.01	0.54±0.01*	0.56±0.01*	0.51±0.01*

\*significantly different from control p≤0.05

The gross examination of F2 pups reported only 1 finding in 1 pup in 1 30 mg/kg litter. The one reported finding was encephalocele. The background incidence seems very low.

**2.6.6.7 Local tolerance** Not reviewed as sponsor is not pursuing an intravenous indication.

### 2.6.6.8 Special toxicology studies

**Study title:** A four week oral immunotoxicity study in the rat.

#### Key study findings:

**Study no.:** IMM0044/ FSRFU-Imm0044-EN-E01

**Conducting laboratory and location:** Sanofi-Synthelabo, Montpellier Cedex, France

**Date of study initiation:** August 25, 2004

**GLP compliance:** statement included

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** SR33589B, batch CL-05754

**Formulation/vehicle:** 0.6% methylcellulose

#### Methods

Sprague Dawley rats(10 males/group) were given an oral suspension of dronedarone at doses of 0, 2, 10 and 50 mg/kg. The doses were the same as those used in the 6-month study.

Cyclophosphamide was used as a positive control. Animals were treated daily for 28 days, until the day before necropsy. All animals were immunized with keyhole limpet hemocyanin (KLH) on day 15 (primary response) and on day 24 (secondary response). Animals were monitored for signs, mortality, body weight, food consumption, hematology, lymphocyte subpopulations, bone marrow cellularity, IgG and IgM levels.

#### Results:

There were no effects upon body weight gain. The hematology showed minimal alterations in PCV. The positive control caused a decrease in WBC and marked effects upon the lymphocyte subpopulations. No clinical signs were reported.

Mean log titer values

	Dose mg/kg				
	0	2	10	50	CP
Anti-KLH IgM titer					
Primary response Titer log	3.23	3.05	3.32	2.93	1.90***
Secondary response Titer log	3.41	3.11	3.17	3.29	1.96***
Anti-KLH IgG titer					
Primary response Titer log	3.05	3.20	2.96	2.78	1.53***
Secondary response Titer log	3.44	3.32	3.29	3.35	2.68***

At the doses used in this study, there were no apparent effects upon the parameters studied. Doses that had caused some kind of clinical signs or alterations in other parameters might have been more informative as to potential immune system effects.

*Evaluation of phototoxicity and/or photoallergy in the guinea pig. PHO121-137-141*

Three studies were conducted to evaluate the phototoxic or photoallergic potential of dronedarone. In the first study, PHO121, a technical problem with the UV lamp made it impossible to complete the experiment. After resolution of the problem the study was started again with the same identified code. The results were presented in this study report. PHO137 was conducted to evaluate the possibility of photosensitization associated with tissue storage. PHO141 was to assess phototoxicity and a dose-response if any.

Ten male albino Hartley guinea pigs per group were given oral doses of 10% gum Arabic vehicle or 0.6% methylcellulose, or 30, 100 or 200 mg/kg of dronedarone. The general experimental design for the 3 studies is summarized below.

Veh or control	1 <sup>st</sup> period(phototoxicity + induction of photoallergy)			Control of phototoxic disappearance	2 <sup>nd</sup> period photoallergy	
	Dose mg/kg	Days of Treatment	Days UV tx	Days UV irradiation w/ no tx	Dose mg/kg	Day tx + irradiation
Lamp contr	-		1,3,7,8,9,10,11	24, 39, 78	-	92
Gum arabic	100	1,3,7,8,9,10,11,14	1,3,7,8,9,10,11	24, 39, 78	30	92
Gum arabic	-				30	92
Lamp contr	-		1,2,28,			58
Gum arabic	-	1,2,3,4,5,6,7	29,		30	58
Gum arabic	30	,8,9,10,13,1	30,31,		30	58
Methylcell	-	4,15,16,20,2	34,35		30	58
methylcell	30	1,22,23,24,27,28,29,30,31,34,35			30	58
Lamp contr	-	-	1	15	-	-
Methylcell	-	1	1			
methylcell	200	1	1			

**PHO121**

Prior to starting the studies the animals were depilated on the right and left flanks. The UV lamp control group received no treatment.

Other animals received 100 mg/kg in 10% gum Arabic for 7 days (day 1,3,7,8,9,10 and 11) over a period of 11 days. Phototoxic effects were assessed on the first day, the following days corresponding to the period of induction of potential photoallergy. The time interval between drug administration and irradiation was 1 hour. Animals received 1 treatment without irradiation on day 14. The right flank was irradiated. The unirradiated left flank served as a control. This procedure was repeated 7 times on each day of drug administration.

### Control of phototoxic effect disappearance

This pertains to persistence of a phototoxic effect. The methods section does not clearly explain what this involved. To quote the report: "...the absence of compound from the animals' organism was controlled after UV irradiation of the skin (without any treatment) on D24, D39 and D78.

Evaluation of photoallergy (second period): The same group of guinea pigs were used for the second period of treatment (day 92) after a treatment free interval of uncertain duration. A group of naïve animals was integrated into the study to serve as control for phototoxicity. Animals were depilated 24 hours prior to irradiation.

#### Treatments:

- UV lamp control with no treatment
- Oral treatment (30 mg/kg) 1 hour prior to irradiation (previously treated at 100 mg/kg first period)
- Oral treatment (30 mg/kg) 1 hour prior to irradiation (drug naïve, phototoxicity Control)

Left flank received UVB spectrum. Right flank received UVA spectrum.

### PHO137(Amiodarone-type protocol)

- Evaluation of phototoxicity and induction of photoallergy (first period)

Animals were depilated.

#### Treatments ( given for 26 days over a period of 35 days):

- UV lamp control, no tx
- Gum Arabic vehicle
- Dosed with 30 mg/kg dronedarone in gum Arabic
- Methylcellulose vehicle
- Dosed with 30 mg/kg dronedarone in methylcellulose

Phototoxicity assessed the first day, with the following days corresponding to the period of induction of potential photoallergy. There was an interval of 1 hour between dosing and irradiation. The left flank was not irradiated, serving as a control. The procedure was repeated 8 days (D1, D2, D28, D29, D30, D31, D34, D35).

- Evaluation of photoallergy (second period)

The same group of guinea pigs were used for the second treatment period (D58) after a drug-free interval.

#### Treatments:

- UV lamp control, no treatment
- 2groups got dronedarone in gum Arabic at a dose of 30 mg/kg ( 1 group served as a phototoxicity control group)
- 2groups got dronedarone in methylcellulose at a dose of 30 mg/kg (1 group served as a phototoxicity control group)

The left flank received UVB irradiation and the right flank received UVA irradiation.

PHO141 Evaluation of phototoxicity and possible test compound storage at high dose (200 mg)

Treatments:

One group received no treatments

Animals received the oral vehicle

Animals received 200 mg/kg

Animals were exposed to UVA and UVB irradiation on the right flank only. The left flank served as another control. Animals were given 1 treatment on day 1. Animals were “allowed to recover” for approximately 15 days.

Day 15: The animals were exposed to irradiation without treatment. The right flank again received UVA and UVB irradiation while the left flank was unexposed.

The animals used in these 3 protocols were examined for clinical signs, body weight, erythema, cutaneous eruption, edema and hyperkeratosis.

## Results

**PHO121 at 100 mg/kg:** After one administration and one irradiation in the UVA and UVB spectra, a phototoxic response was noted in 8/10 animals. During the first period, the phototoxic response was observed during the 6 days of treatment and irradiation. It's not entirely clear how much time without drug the animals were given, but from table (6.2)1 that after a drug clearance phase, several animals were still showing positive reactions to UV irradiation.

Day 25-D40: 0/10 lamp controls had erythema compared to 4/10 drug-treated animals day 25 and 3/10 drug-treated animals day 40.

**PHO137 at 30 mg/kg:** After 24 days of treatment and 8 days of irradiation, response were not observed which supported a cumulative effect of the drug.

**PHO141 at 200 mg/kg:** After single treatment and irradiation a phototoxic response was noted in 8/10 animals.

**Control of Phototoxic effect disappearance: performed for animals of studies PHO121 and PHO141 as no phototoxic effect was apparent in study PHO137.**

**PHO121:** animals treated at 100 mg/kg during the first period

Day 25: grade 1 reaction in 4/10 guinea pigs

Day 40: grade 1 reaction in 3/10 guinea pigs

Day 79: no reaction reported for any of the guinea pigs

**PHO141:** animals treated at 200 mg/kg during the first period.  
The phototoxic effect was controlled 15 days after cessation of treatment.

The sponsor described the erythematous reactions as being no different between 100 mg/kg and 200 mg/kg doses of dronedarone. The sponsor also makes the comment that the reactions seen in this study cannot be compared to those observed during the study with amiodarone. However, the amiodarone data was not available in the study report and so can't be evaluated. It would have been most helpful to have a side by side comparison of the two drugs, including their UV absorption spectra. The reactions noted were graded 1-2 on a scale of 0-4. Zero was described as no reaction. One was the minimal erythema dose and 2 was well-defined erythema, pale pink.

### Study of the hemolytic potential in vitro. HEM0009

Freshly collected baboon blood (*Papio ursinus*), 0.4ml, was incubated at 37°C with 0.1 ml test solution for 30 minutes or for 2 hours. Concentrations of drug used were (mg/ml blood): 0.666, 0.333, 0.166, 0.083, 0.041, 0.02 and 0.01. Replicates were not processed. Saline was used as the negative control with saponin as the positive control. The vehicle for the study was 0.01N acetate buffer and 40% PEG400.

Hemolysis % was described as (RBC saline – RBC treated)/(RBC saline). I did not find any description of a standard curve for assessment of how accurate the estimates of hemolysis are.

#### HEMOLYSIS AFTER 30 MINUTES OF INCUBATION

COMPOUND	mg/ml blood	Hemoglobin (g/l)	Erythrocytes ( $10^6/\text{mm}^3$ )	Hemolysis (%)	Hematocrit (%)	MCV ( $\text{micro}^3$ )
SALINE	0	110	4.63	0	34	73.4
VEHICLE	0	108	4.51	2.6	35	77.6
SAPONIN	0.5	108	2.75	40.6	22	80
	0.2	109	4.29	7.3	36	83.9
SR33589B	0.666	113	0.04	100	0	
	0.333	112	0.44	90.4	Unreadable	
	0.166	107	2.92	36.9	Unreadable	
	0.083	109	4.38	5.3	35	79.9
	0.041	109	4.51	2.6	36	79.8
	0.02	109	4.54	1.9	36	79.3
	0.01	109	4.54	1.9	36	79.3

Results were similar after the 2 hour incubation. Concentrations of drug  $\geq 0.166$  mg/ml of blood caused hemolysis equal to or greater than that observed in the presence of 0.2 mg/ml saponin.

**RBC compatability:**

Two studies were performed using a saponin comparison but not distilled water.

**Hemolytic potential in vitro HEM013**

Baboon blood (0.4ml) was incubated at 37°C with 0.1ml test solution for 30 minutes or for 2 hours. The concentrations tested were 0.2, 0.1 and 0.05 mg/ml blood.

Negative control: saline.

Positive control: saponin

Vehicle: acetate/acetic buffer

## Summary of hemolysis

Compound	Mg/ml blood	Hemolysis %	
		After 30 minutes	After 2 hours
Saline	0	0	0
vehicle	0	2.6	5
saponin	0.2	2	4
	0.5	44.6	73
SR33589B	0.2	4.6	5
	0.1	0.04	1
	0.05	0	0.7

This study showed that under the conditions used, undiluted formulation of dronedarone induced a hemolytic effect similar to that of the vehicle. This finding did not hold in the in vivo studies.

*Study of the hemolytic potential in vitro. HEM0015*

In this study, 0.4 ml of human blood was incubated at 37°C with 0.1 ml test solution for 30minutes or 2 hours. The concentrations of dronedarone tested were 0.2, 0.1 and 0.05 mg/ml blood.

## Controls:

Saline

Saponin

Mannitol

Monosidic anhydrous phosphate

Water for injections

Compound	Mg/ml blood	Hemolysis %	
		After 30 minutes	After 2 hours
Saline	0	0	0
Vehicle	0	0.2	3.3
saponin	0.5	57.8	61.1
	0.2	6.1	3.7
SR33589B	0.2	1.4	5.1
	0.1	0.9	1.4
	0.05	0.5	1.2

At 30 minutes of incubation, the hemolysis seen with the drug slightly exceeded that achieved with vehicle alone. After 2 hours of incubation, only the highest dose of drug (undiluted formulation) induced more hemolysis than did the vehicle. The sponsor felt that the hemolysis reported was due only to the excipient.

### Study of hemolytic potential in vitro. HEM0026

Human blood, 0.8 ml, was incubated at 37°C with 0.2 ml test solution for 30 minutes or 2 hours. The concentrations tested were 0.8, 0.4, 0.2, 0.1 and 0.05 mg/ml blood. The vehicle contained hydroxypropyl  $\beta$ -cyclodextrin, D-mannitol, sodium phosphate monobasic and water for injections.

Controls:  
Saline  
saponin

#### Summary of hemolytic effects

compound	Mg/ml blood	Diluent	Hemolysis %	
			After 30 minutes	After 2 hours
Isotonic solution	0			
5% glucose serum	0		0	0
Saponin	0.2	EPPI	0	0
	0.5	EPPI	82.7	90.9
Undiluted vehicle	0		0	0
Vehicle dil 1:2	0	Glucose	0	0
SR33589B	0.8		0	1.0
	0.4	glucose	1.0	2.0
	0.2	glucose	0	0
	0.1	glucose	0	0
	0.05	glucose	0	0

Under the conditions of the study, there was slight increase in hemolysis after 2 hours in the 2 highest concentrations of drug. The sponsor attributed this to imprecision in the measurement. In neither of the in vitro hemolysis studies do we see standard curves to assess the range of measurement of the assay. Saponin was included at 2 concentrations but distilled water was not included as the standard positive control.

**2.6.6.9 Discussion and Conclusions**

**2.6.6.10 Tables and Figures**

**2.6.7 TOXICOLOGY TABULATED SUMMARY**

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

**OVERALL CONCLUSIONS AND RECOMMENDATIONS**

Conclusions: 1) Dronedarone appears to have an undescribed endocrine effect  
2) the drug is teratogenic  
3)the drug appears to have related carcinogenicity  
4) there is a poorly defined effect upon the thyroid gland  
5) Target organs of toxicity include the liver, kidneys, gastrointestinal tract and female reproductive tract

Unresolved toxicology issues (if any): 1) Studies are needed for qualification of the impurity SR194090 2) If the sponsor wishes to claim that the mammary tumors are due to a mechanism that is irrelevant to humans, they will need to provide mechanistic data 3)The mechanism by which dronedarone disrupts female cyclicity 4) The receptor binding profile of dronedarone and the major metabolites needs to be investigated. 5) Effect on thyroid hormone turnover.

Recommendations: Approvable based upon clinical considerations and resolution of these issues.

Suggested labeling: See Section I.

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

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PHARMACOLOGIST