CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-425

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
NDA: 22-425
Submission date: June 26, 2008
Drug: dronedarone hydrochloride (Multaq)
Sponsor: Sanofi
Indication: atrial fibrillation or atrial flutter

Reviewing Division: Division of Cardiovascular and Renal Products

Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted for dronedarone hydrochloride to be sufficient to support approval for the indication as proposed above.

Carcinogenicity:
Two year oral gavage carcinogenicity bioassays were conducted with dronedarone hydrochloride in rats and mice. Histiocytic sarcomas in male mice, mammary adenocarcinomas in female mice and hemangiomas in male rats were considered to be drug-related by the executive carcinogenicity assessment committee. The reviewer has noted that these occurred at doses that produced AUCs that were 5 to 8 times the clinical AUC at the maximum recommended human dose. As noted by the reviewer, the mammary tumors may be related to an increase in prolactin. The sponsor provided studies demonstrating an increase in prolactin in mice at the high dose used in the carcinogenicity study. It is not clear if prolactin is elevated in human subjects taking dronedarone hydrochloride. It is appropriate to describe these tumors in the labeling as suggested by the reviewer.

Developmental and Reproductive toxicity:
Dronedarone is teratogenic in rats at doses similar to the recommended human dose. Consequently, the sponsor proposes that dronedarone be contraindicated in pregnant women. This is appropriate.

Other issues:
Amiodarone, which is pharmacologically and structurally related to dronedarone, can produce perturbed thyroid function. While the absence of iodine in the dronedarone structure might be expected to produce fewer thyroid effects than amiodarone, some alterations in thyroid hormones were observed in some of the toxicology studies. However, these changes were not particularly profound and appeared to be much less in magnitude than those produced by amiodarone in a rat study in which the two compounds were compared. Thyroid hormones were monitored in clinical studies so a more definitive assessment would be provided from the clinical data.

The reviewer notes that the applicant did not submit complete receptor binding studies for the parent drug and main metabolites. The sponsor did provide some information on binding to dopaminergic receptors and referred to literature information indicating

inhibition of the thyroid hormone receptor-α1. This was in addition to studies associated with the possible primary pharmacology of dronedarone at sodium, potassium and calcium channels and activity at α1, ß1 and ß2 adrenergic receptors. While it is true that large receptor screens are often conducted early in drug development, it is not clear that this information would be particularly useful at this stage when other studies that would identify any secondary pharmacology of concern have already been completed. If the sponsor was attempting to describe a particular mechanism for one of the observations based on some unexpected receptor interaction then perhaps targeted studies of such an interaction would be informative. It does not appear that further general receptor binding data would be necessary at this point to support approval of the drug.

Conclusions:
I concur with the Division pharm/tox conclusion that the nonclinical data support approval of this NDA. I do not think additional nonclinical studies are needed.

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/s/

Paul Brown
4/30/2009 01:17:03 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-425**
DOCUMENTS REVIEWED:	**complete response to non-approvable letter**
DATE RECEIVED BY CENTER:	**June 26, 2008**
PRODUCT:	**Multaq® (dronedarone hydrochloride tablets)**
INTENDED CLINICAL POPULATION:	**patients with atrial fibrillation or atrial flutter**
SPONSOR:	**Sanofi**
REVIEW DIVISION:	**Division of Cardio-Renal Drug Products**
PHARM/TOX REVIEWER:	**Elizabeth Hausner, D.V.M.**
PHARM/TOX SUPERVISOR:	**Charles Resnick, Ph.D.**
DIVISION DIRECTOR:	**Norman Stockbridge, M.D., Ph,D.**
PROJECT MANAGER:	**Russell Fortney**

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EXECUTIVE SUMMARY

Background

Dronedarone is a benzofuran derivative structurally similar to amiodarone and demonstrating electrophysiologic characteristics of all 4 Vaughan-Williams classes of anti-arrhythmic compounds. Dronedarone was originally presented in NDA 21913 for treatment of atrial fibrillation (AF) or atrial flutter (AFL).

The Division issued a "non-approvable" letter for NDA 21913, for both clinical and non-clinical reasons. The clinical reasons for non-approval included an unfavorable risk: benefit relationship. The potential symptomatic improvement offered by the drug was also weighed against the nonclinical issues of carcinogenicity, teratogenicity, and endocrine effects (thyroid gland and female cyclicity). The complete response to these concerns was filed under NDA 22425 with a new indication of treating patients with AF/AFL or with a history of such events to reduce the risk of cardiovascular hospitalization or death. The proposed dose is 400 mg twice a day.

Summary of Non-Clinical Concerns

Endocrine Effects

Thyroid effects: Because of dronedarone's similarity to amiodarone, thyroid hormone levels were measured in several toxicology studies. There were changes in circulating hormone levels, sometimes statistically significant, in both rats and dogs. Thyroid tumors were not seen in the carcinogenicity study. The findings were discussed in detail in the review for NDA 21913.

Reproductive toxicology: The sponsor and I agree that the drug is teratogenic. This is a labeling issue. Another issue was the apparent effect on female cyclicity. This was manifested in a number of toxicology studies in which the drug-treated females were paused in diestrus. Other studies showed an increase in the frequency of irregular cycles and lack of cycles. Ovarian and uterine weights were affected in several studies. These findings were discussed in detail in the review for NDA 21913.

No non-clinical material was submitted to address the endocrine issues. According to the Clinical Safety Summary, the sponsor gathered clinical data to assess thyroid hormone effects of dronedarone. This data is in the clinical section of this submission and will be addressed by the medical officer.

Carcinogenicity

The Exec CAC considered the following findings to be drug-related: histiocytic sarcoma in male mice, mammary adenocarcinomas in female mice and hemangiomas in male rats. The minutes of the Executive CAC meeting are on file under IND 49484.

The sponsor's Clinical Safety Summary for NDA 22425 contains the following comments on the carcinogenicity studies:

- A slight increased incidence of adenocarcinoma was observed in the mammary gland of female mice, at the highest dose only, with no effect in male mice or in rats of either sex. The slightly raised incidence is associated with a modest increase in prolactinemia, explaining the small mammary gland response. In addition, the tumors themselves are those classically noted for the mouse, and are histologically different from those in humans. This mechanism of tumors is acknowledged to be specific to rodents with no relevance to humans.
- A slight increase in histiocytic sarcoma was observed in mice at the highest dose level, but not in rats of either sex. The low incidence lies within historical values of the National Toxicology Program, and there was no increase in the frequency of other lymphoreticular tumors in both carcinogenicity studies. In addition, no analogous tumor is known to occur in man.
- Dronedarone induced an increased incidence of benign vasoproliferative lesions in the mesenteric lymph nodes in rats and female mice, at the highest dose only. In the literature, venous or lymphatic obstruction is known to lead to vascular transformation of lymph nodes. Dronedarone has been shown to accumulate preferentially in the mesenteric lymph nodes and to induce locally accumulation of foamy macrophages in rats, a species particularly susceptible to this type of effect. The physical presence of foamy macrophages in the mesenteric lymph nodes could be sufficient to alter local blood flow and thus induce vascular lesions. Hemangiomas are not precancerous changes and do not transform into malignant hemangiosarcomas in either animals or man. Both lesions are extremely rare in humans at the level of lymph nodes. Overall, the proliferative vascular changes seen in the rat are considered to be reactive-proliferative, rare in occurrence and innocuous, therefore not relevant for man.

To support their assertion of a lack of clinical significance of the carcinogenicity findings, the sponsor submitted two studies measuring circulating prolactin levels in mice following dronedarone administration, an immunohistochemistry study to show differences in blood flow to mesenteric lymph nodes (for reasons noted in the sponsor's statement above) and an autoradiography study that focused on the amount of drug-associated radioactivity found in lymph nodes.

Neither study designed to demonstrate altered blood flow supported that hypothesis. It was reported that the tumor incidences seen in the dronedarone studies were within the background range of the NTP studies reported in Haseman et al. The referenced publication is Haseman et al. "Use of dual control groups to estimate false positive rates in laboratory animal carcinogenicity studies." Fundamental and applied Toxicology: 573-584 (1986). There is a question of the relevance of the Charles River SD rats and CD-1 mice used in the historical studies to the SD(IOPS Caw) rats (different strain) and the CD-1 mice (genetic drift from time and geography) used almost 20 years later.Slightly

more recent databases from Charles River do indicate that that the incidences of hemangioma, hemangiosarcoma and histiocytic sarcoma in the dronedarone studies were within historical control ranges albeit for SD rats.

Increased prolactin was hypothesized to be the cause of the mammary tumors reported in the mice and presented as a mechanism irrelevant to humans. The sponsor showed increases in serum prolactin levels in the mice that persist for at least 28 days. The total duration of elevated prolactin is not clear. Antipsychotic drugs that increase prolactin in rodents via binding to the D2 dopaminergic receptor have also been shown to increase prolactin in humans.

The sponsor has shown only modest binding (IC50>10 μM) of dronedarone to the D2 dopaminergic receptor. No information was provided for receptor binding of the major metabolites.

The sponsor did not demonstrate in the non-clinical section of the submission that prolactin does not increase in humans treated with dronedarone.

The current published clinical literature has numerous articles discussing epidemiologic and other data that support increased prolactin as a factor involved in carcinogenesis. The sponsor's proposed mechanism for the mammary tumors can't be dismissed out of hand as irrelevant to human health.

The questions of alteration of thyroid homeostasis and effects on female cyclicity were reported to have been addressed clinically and as such will be evaluated by the medical officer.

Recommendations

A. Recommendation on approvability: From a non-clinical view, the drug could be approved despite the lack of data to support the carcinogenicity findings as not clinically important. This depends upon the evaluation of the risk: benefit relationship in view of the new indication.

B. Recommendations for additional nonclinical studies: 1) receptor binding studies for the parent drug and main metabolites, and 2) data to show that the tumors are not clinically relevant, although this may not necessarily be non-clinical data.

C. Recommendations on labeling

Under "**NONCLINICAL TOXICOLOGY, Carcinogenesis, Mutagenesis, Impairment of Fertility,**"

the statement :

> "In 2 year-oral carcinogenicity studies, the highest dronedarone dose administered for 24 months was 70 mg/kg/day in rat and 300 mg/kg/day in mice.
> Observations were increased incidence of mammary gland tumors in female mice, histiocytic sarcomas in mice and hemangiomas at the mesenteric lymph node level in rats, all at the highest tested dose only (corresponding to an exposure of 5 to 10 times that of the human therapeutic dose). Hemangiomas are not precancerous changes and do not transform into malignant hemangiosarcomas in either animals or man.
> None of these observations was considered relevant for humans."

should be replaced with :

> "In studies in which dronedarone was administered to rats and mice for up to 2 years at doses of up to 70 mg/kg/day and 300 mg/kg/day, respectively, there was an increased incidence of histiocytic sarcomas in dronedarone-treated male mice (300 mg/kg/day or 5X MRHD based on AUC comparisons), mammary adenocarcinomas in dronedarone-treated female mice (300 mg/kg/day or 8X MRHD based on AUC comparisons) and hemangiomas in dronedarone-treated male rats (70 mg/kg/day or 5X MRHD based on AUC comparisons). "

The statement:

"Dronedarone had no genotoxic effects, based on one *in vivo* micronucleus test in mice and four *in vitro* tests: the Ames test with or without metabolic activation, a DNA repair test on rat hepatocytes, a gene mutation assay on hamster fibroblasts and a cytogenetic study of human lymphocytes."

should be replaced with:

"Dronedarone did not demonstrate genotoxic potential in the *in vivo* mouse micronucleus test, the Ames bacterial mutation assay, the unscheduled DNA synthesis assay, the HPRT gene mutation assay in Chinese Hamster V79 fibroblasts and an *in vitro* chromosomal aberration assay in human lymphocytes."

The statement :

"Dronedarone was not shown to alter fertility in animal studies up to 100 mg/kg/day"

should be replaced with:

" In fertility studies conducted with female rats, Dronedarone given prior to breeding and implantation caused an increase in irregular estrus cycles and cessation of cycling at doses ≥10mg/kg (equivalent to 0.12X the MRHD on a mg/m^2 basis)." Corpora lutea, implantations and live fetuses were decreased at 100 mg/kg (equivalent to 1.2X the MRHD on a mg/m^2 basis). There were no reported effects on mating behavior or fertility of male rats at doses of up to 100 mg/kg/day.

Under **"Pregnancy,"**

the paragraph that reads:

"MULTAQ can cause fetal harm when administered to pregnant women. In rats, dronedarone caused marked effects on embryo-fetal development at 100 mg/kg/day such as increased post-implantation losses, reduced fetal and placental weights and various external, visceral and skeletal malformations in most fetuses. At lower dosages, up to 50 mg/kg/day (corresponding to 4.5 times the recommended human therapeutic dose), dronedarone had no effects on the litters (with the exception of a transient minor effect on the bodyweight gain of the pups from D1 to D4 post-partum). Dronedarone had no adverse effects on the mothers and their litters up to 30 mg/kg/day. In rabbits, the high dose level (200 mg/kg/day, equivalent to 5X the MRHD on a mg/m^2 basis) did not induce any effects to fetuses."

Should be modified to read as follows:

> "Dronedarone was teratogenic in rats given oral doses ≥80 mg/kg (a dose equivalent to the maximum recommended human dose on a mg/m^2 basis), with fetuses showing external, visceral and skeletal malformations (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, ectrodactylia, syndactylia, anterior and/or posterior club feet). In rabbits, dronedarone caused an increase in skeletal abnormalities (anomalous ribcage and vertebrae, pelvic assymetry) at doses ≥20 mg/kg (the lowest dose tested and approximately half the MRHD on a mg/m^2 basis).

The section **13.2 Animal Toxicology and/or Pharmacology** is unnecessary and should be removed.

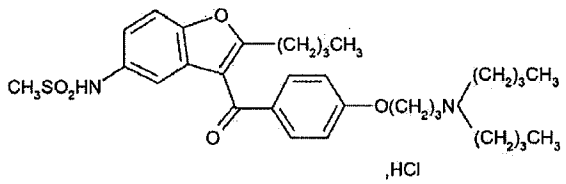
PHARMACOLOGY/TOXICOLOGY REVIEW

NDA number: 22425
Sponsor: Sanofi-Synthelabo
Agent: NA
Submission Type: complete response to non-approvable letter
Date of Submission: June 27, 2008
CDER Stamp Date: electronic submission

Reviewer: Elizabeth Hausner, D.V.M.
Division: Division of Cardio-Renal Drug Products
HFD #: 110
Review Completion Date: December 9, 2008

Drug:

Trade name: Multac®
Generic name: dronedarone
Code name: SR33589B
Chemical name:N-{2- butyl- 3-[4-(3- dibutylaminopropoxy) benzoyl] benzofuran- 5- yl}methanesulfonamide, hydrochloride.
CAS registry number: 141626-36-0
Molecular formula/molecular weight: $C_{31}H_{44}N_2O_5S,HCl$/ 593.2
Structure:



Pharmacologic Class: antiarrhythmic

Related INDs/NDAs/DMFs: Sanofi-Winthrop IND 49484
Sanofi-Synthelabo NDA 21913

Intended Clinical Population: patients with atrial fibrillation or atrial flutter for the reduction of the risk of cardiovascular hospitalization or death (new indication)

Clinical Formulation: 400 mg of dronedarone base with inactive ingredients of hypromellose, starch, crospovidone, poloxamer 407, lactose monohydrate, colloidal silicon dioxide, magnesium stearate

Route of Administration: oral

Prolactin Assay After a Single Oral Administration to Mice

Report number: DIV1142
Study location: Sanofi-Aventis Recherche and Developpement, Montpellier Cedex, France
GLP: statement included
QA: yes
Study dates: in-life started September 12, 2007
Test article: SR33589B, batch number CL-05754, purity 99.9% by HPLC
The chemical analysis form lists the potency of active drug as 93.42%
A conversion factor of 1.066 was used to correct for the salt form.
Animals: female mice, CD-1(ICR), 6-8 weeks of age at initiation of dosing

SR33589B was orally administered as a suspension in 0.6% methylcellulose aqueous solution to 20 female mice in a single gavage dose of 300 mg/kg/day, the high dose used in the mouse carcinogenicity study. A control group of females received the vehicle of methylcellulose (0.6% aqueous solution). For 9 days prior to the study the animals were habituated to gavage dosing (with water) and being placed in the anesthesia box.

Un-fasted animals were anesthetized with inhalant isoflurane, approximately 2 hours after drug administration (based on the Tmax in mice occurring at 2 hours). The blood was collected, the serum frozen and sent to Anilytics, Inc., Gaithersburg, MD. Animals were euthanized immediately after blood collection. The prolactin was measured by a radio-immunoassay (RIA) method. Prolactin concentrations in the test samples were determined from a standard curve generated under the same conditions. It was reported to be a species specific assay but no validation material was presented or referenced. No information was provided regarding the standard curve or the variability of the analysis itself.

Observations

- Mortality: twice a day and only once on the day of termination
- Clinical signs: at least once on day of dosing
- Serum prolactin levels at time of euthanasia.

Results

There was no unscheduled mortality. No clinical signs were reported.

There was a difference in the mean and median values for the control and treated groups that was significant at $p<0.001$ by the exact two-sided Wilcoxon test.

Table 1 - Descriptive statistics

Analysis Variable : Prolactin (Ng/mL)				
Dose (mg/kg)	**N Obs**	**Mean**	**Median**	**Std Dev**
0	20	52.78	37.66	42.93
300	20	211.10	195.07	69.22

Because hormonal assays are subject to profound variability, I have included in this section a copy of the sponsor's box plot of the results as well as the numerical description.

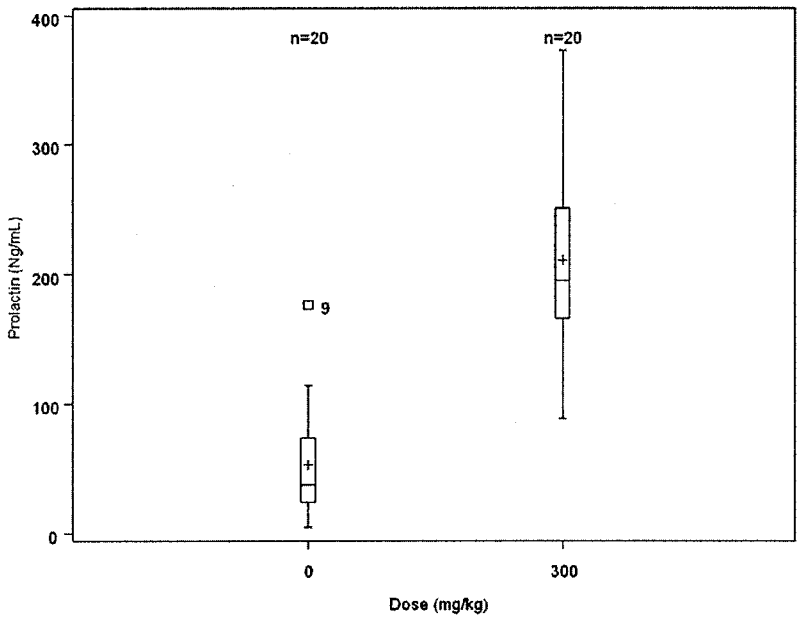


Figure 1 - Boxplots of the Prolactin per group

The means (+) are greater than the medians for both groups, that indicates a dissymmetry in the distributions. There is also an animal (n°9) that obtains a high value (177 Ng/mL) compared to its group results.

Prolactin Assay After a 28-day Oral Administration to Mice

Report number: DIV1123
Study location: Sanofi-Aventis Recherche and Developpement, Montpellier Cedex, France
GLP: statement included
QA: yes
Study dates: study started September 5, 2007
Test article: SR33589B, batch number CL-05754, purity 99.9% by HPLC
The chemical analysis form lists the potency of active drug as 93.42% and a "purity correction" of 93.80 is listed. A conversion factor of 1.066 was used to correct for the salt form.
Animals: female mice, CD-1(ICR), 6-8 weeks of age at initiation of dosing

SR33589B was orally administered as a suspension in 0.6% methylcellulose aqueous solution to 20 female mice at 300 mg/kg/day for 28 days. A control group of females (n=20) received the vehicle. Two hours after the administration on day 28, the animals were anesthetized, blood collected for serum analysis and the mice euthanized. The 300 mg/kg dose was chosen as it was the high dose of the mouse carcinogenicity study.

The sponsor also detailed procedures to minimize stress on the animals:

> Note: For the last 10 days of treatment, to minimize stress on the actual day of blood sampling, mice of both groups were daily administered with distilled water (0.25 mL) and placed in the anesthesia box for approximately 2 minutes for habituation.

It is unclear to me why the animals were gavaged with water as they already had at least 18 days of experience with daily gavage. The sponsor did not describe what steps were taken to minimize circadian/diurnal variation in the hormone assays.

Observations

- Mortality: twice daily and once on day of termination
- Signs: at least once a day
- Body weight: pre-study, then weekly
- Prolactin: Day 28, approximately 2 hours after drug administration, the mice were anesthetized with inhalant isoflurane. Blood was collected and frozen. The frozen samples were sent to Anilytics, Gaithersburg, MD. The serum was analyzed for prolactin using a radio-immunoassay (RIA). It was reported to be a species specific assay but no validation material was presented or referenced.
- Necropsy: Necropsy was performed only for mice found dead or euthanized moribund.
  This was reported to include macroscopic examination, preservation of grossly abnormal tissues and limited histological evaluation. The tissues collected are listed below.

Organ and tissues taken

Liver	Uterine horns/Cervix/Vagina
Spleen	Esophagus
Gallbladder	Stomach (non glandular)
Kidneys	Stomach (glandular)
Adrenal glands	Duodenum
Thymus	Jejunum
Heart	Colon
Lungs	Brain
Ovaries/Oviducts	Rectum (36F only)

Results

There was no unscheduled mortality in the control group. Two females from the treated group died prior to euthanasia. Female 28F showed signs of hunched posture on day 7 as well as 20% weight loss compared to her pre-study value. She appeared weak, cold to the touch and was recumbent by day 8. She was euthanized Day 8. According to the text, there were no significant gross lesions and the microscopic lesions suggested acute to chronic stress and under-nutrition. Histologic changes to support that were reported for the spleen, adrenals, thymus, brown fat and pancreas. There was more detail regarding the histologic findings in the appendices of the report. Something not mentioned in the main text was the presence of cardiac degeneration. The sponsor felt that the cause of death could not be determined. The other female, 36F, was found dead on day 17 without any premonitory signs reported. It was also reported that there were minimal gross or histologic changes which did not explain the cause of death. The appendix reported necrotizing pneumonia for female #36, also not mentioned in the main text. It was reported that the remaining animals did not display any clinical signs.

There were statistically significant differences in the rate of weight gain in the treated animals.

Prolactin assay after a 28-day oral administration to mice

Summary of Body Weight Changes

Sex = FEMALE

Dose mg/kg/d		Day (-1)-7	Day (-1)-14	Day (-1)-21	Day (-1)-27
Group 1	MEAN	0.68	0.61	1.23	1.57
0	STD	1.22	1.34	1.75	2.03
	N	20	20	20	20
Group 2	MEAN	1.35 NS	1.85 *	3.10 *	2.88 *
300	STD	2.15	1.60	1.58	1.38
	N	20	19	18	18

There was a statistically significant difference between the treated and control group median prolactin levels (Exact two-sided Wilcoxon p <0.001).

	Prolactin level (ng/mL)			
Dose (mg/kg)	N Obs	Mean	Median	Std Dev
0	20	81.13	68.46	60.29
300	18	190.61	193.49	64.64

The box and whisker plot is also shown for clarity.

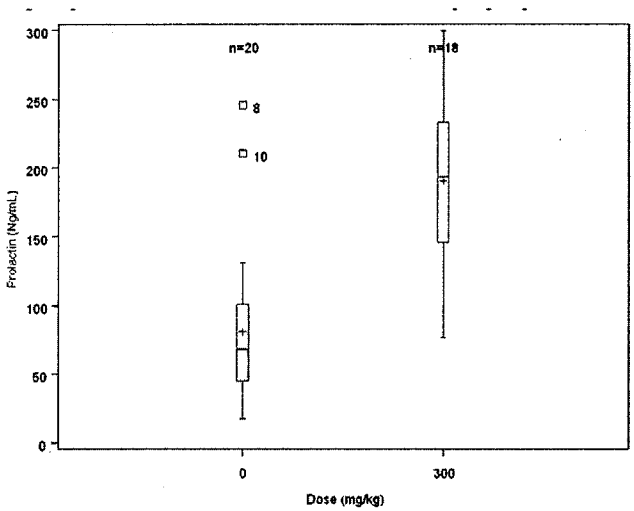


Figure 1 - Boxplots of the Prolactin per group

In control group, the mean (+) is greater than the median, that indicates a dissymmetry in the distributions. There are also two animals (n°8 and n°10) that obtain high values (respectively 245.76 Ng/mL and 210.48 Ng/mL) compared to their group results.

Study of the Potential Alteration of Blood Flow in Mesenteric Lymph Nodes after Repeated Oral Administrations of SR33589B to Female Sprague-Dawley Rats.

Study number: 070164
Report number: DIV1124
Study location: Oncodesign, Dijon Cedex, France
Study dates: initiated November 15, 2007
GLP: statement not found
QA: no
Test article: SR33589B batch CL-07444, purity 94.53%
Diltiazem batch 7Y05431, purity 99.8%
Vehicle: 0.6% aqueous methylcellulose solution
Animals: female Sprague-Dawley rats

The purpose of this study was to analyze the possible alteration of blood or lymph flow in mesenteric lymph nodes induced by repeated oral administration of dronedarone to female SD rats. Diltiazem was used "as a vasodilator that would potentially produce changes in the blood supply to the mesenteric lymph nodes."

Sixty healthy female SD rats were assigned to 3 groups of 20 rats according to body weight. The treatment schedules were:

- Group 1: 20 rats received 1 daily oral administration of vehicle for 28 consecutive days
- Group 2: 20 rats received 1 daily oral administration of SR33589B at 100 mg/kg/day for 28 days
- Group 3: 20 rats received 1 daily oral administration of diltiazem at 100 mg/kg/day for 28 days

Blood volume in mesenteric lymph nodes was studied by detecting a hydrophilic dye (Patent Blue V) using HPLC/UV analysis. The change in quantity of Patent Blue V in mesenteric lymph nodes could reflect a change in blood volume in the lymph nodes.

Functionality and permeability of the lymph node blood vessels was determined with two dyes: Hoescht and dextran-FITC (fluorescein isothiocyanate linked to a specified size of dextran).

Density of vessels was evaluated by detecting the RecA-1 antigen expressed on rat endothelial cells by immunohistochemical methods. Detection of CD68 antigen (a glycosylated protein expressed mostly on macrophages) was used to determine macrophage infiltration in lymph nodes.

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

Group	No. rats	Test substance	Treatment dose (mg/kg/day)	Adm. route	Volume of administration (mL/kg)	Number of rats included in the assessment of mesenteric lymph nodes functionality	
						Patent Blue V level determination	RecA-1 and CD68 immunodetection
1	20	Vehicle	-	PO	5	10	10
2	20	SR33589B	100	PO	5	9	9
3	20	diltiazem	100	PO	5	9	9

The rats were anesthetized, the mesenteric lymph nodes exteriorized and Patent Blue V was injected into the tail vein. Determination of Patent Blue V uptake was done by extracting the dye from the lymph nodes and assessing by HPLC/UV methods using an internal standard (tartrazine).

The other designated rats were anesthetized, the mesenteric lymph nodes exteriorized and the rats given IV boluses of Hoecsht dye followed 30 seconds later by Dextran-FITC. The lymph nodes for each animal were embedded in the same block for analysis. For each block (each animal), 3 slides were prepared. Each set of 3 slides was analyzed as summarized by the sponsor below:

Section	Marker	Evaluation	Technique
1	Hoechst dye / Dextran-FITC dye	Blood perfusion and vessels permeability (5) / Effective vascular volume and functional vessels (6)	Fluorescence: blue / Fluorescence: green
2	RecA-1	Vessel density	Immunohistochemistry
3	CD68	Macrophages detection	Immunohistochemistry

Vessel permeability and functionality assessment: pictures of the lymph nodes were taken with a fluorescence microscope. The images were converted to a binary black and white image followed by objective quantification with a computer program.

Vessel density determination by immunohistochemistry: RecA-1 detection
Immunohistochemical (IHC) procedures were followed. For each section, a single area was analyzed (semi-quantitative analysis). One representative pictures was taken for each rat.

Macrophage infiltration detection by immunohistochemistry: CD68 detection
Standard IHC techniques were used. For each section, a single area was analyzed (semi-quantitative analysis). One representative picture was taken per rat.

Analysis of RecA-1 and CD-68 immunodetection: Blindly analyzed by a consultant pathologist for Oncodesign

A grade (semi-quantitative analysis) was determined for each slide using the following scale:

- 0: no staining.
- 1: low staining.
- 2: low to intermediate staining.
- 3: intermediate staining.
- 4: strong staining.
- 5: very strong staining.

For each group the mean grade was calculated.

For the detection of vessels in mesenteric lymph nodes, the percentage of post-capillary veins was also determined.

For the detection of macrophages in mesenteric lymph nodes, a grade was determined for the cortical and for the medullary zones.

Other Observations

Moribundity, clinical signs and behaviour: each day
Body weights: twice a week
Necropsy: gross examination of heart, lungs, liver, spleen, kidneys and gastro-intestinal tract.

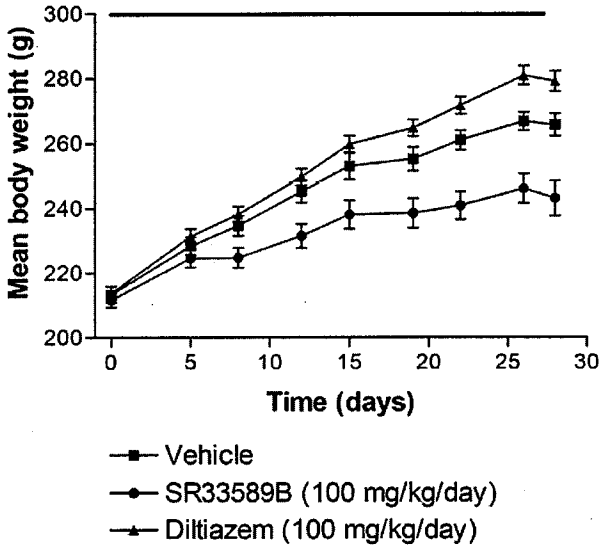
Results

Signs and Mortality

Two rats treated with SR33589B were found dead, one on Day 4 and the other on Day25. Two rats treated with diltiazem were found dead on Day 5 and day 6. The only significant necropsy findings reported for these animals were liquid in the lungs. The sponsor felt that the deaths were due to gavage accidents rather than directly due to test articles. No treatment related macroscopic changes were reported for any of the examined organs.

Body weight

All of the treatment groups showed mean weight gains. The rats treated with ditiazem showed the greatest rate of gain followed by the control group. Animals treated with SR33589B showed the lowest mean body weight gains.



Reviewer's Summary of Body Weight Gains

Day	vehicle	SR33589B 100 mg/kg/day	Diltiazem 100 mg/kg/day
0	213.4±11.5	211.6±9.9	213.7±9.4
12	245.2±15.4	231.5±16.2	249.7±10.8
28	265.7±15.8	243.1±22.8	279.1±13.4
Δday28-day0	52.3	31.5 (-40%)	65.4(+25%)

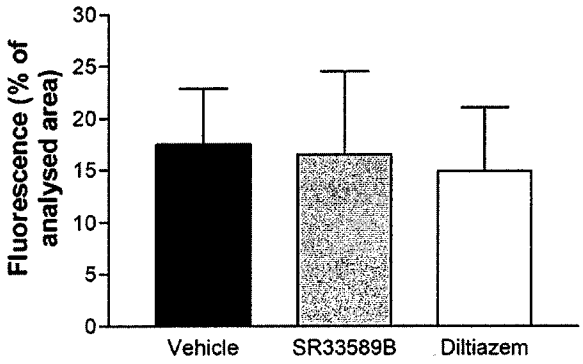
Numbers in parentheses are percent difference from control

Patent Blue V (HPLC-UV analysis)

The mean Patent Blue V quantity in mesenteric lymph nodes in rats treated with dronedarone and diltiazem was not statistically different from rats receiving the vehicle.

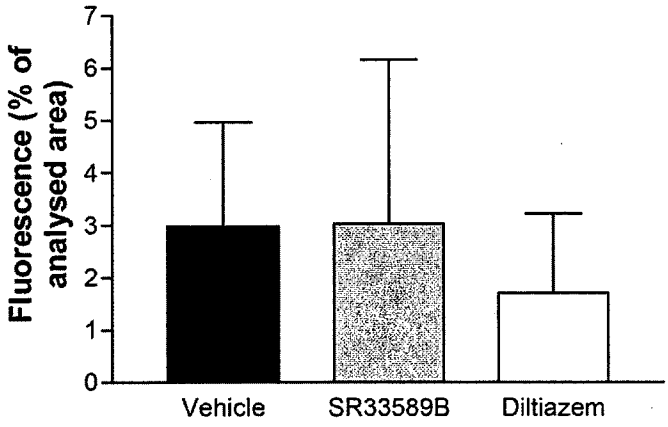
Hoecsht dye (perfusion)

There was no significant difference in the fluorescence detected in the analyzed areas of the mesenteric lymph nodes.



Dextran-FITC ("functionality")

There is a slight decrease in mean fluorescence for the diltiazem group but there are no significant differences between the different treatment groups.



The Hoecsht and Dextran-FITC results are consistent with each other, indicating that dronedarone had no influence on the functionality and permeability of the blood vessels.

RecA-1: vessel density

There was no significant difference in percentage of post-capillary veins between treatment groups.

Individual and mean±SD grade determined for vessel density assessment in mesenteric lymph nodes

vehicle	SR33589B 100 mg/kg/day	Diltiazem 100 mg/kg/day
3.1±1.1	3.3±0.5	2.7±0.7

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Individual and mean ±SD proportions of post capillary veins determined in mesenteric lymph nodes

vehicle	SR33589B 100 mg/kg/day	Diltiazem 100 mg/kg/day
42.0±15.6	51.6±12.5	53.4±10.6

CD68: macrophage infiltration

The sponsor presented mean±SD grade determined for the assessment of macrophage infiltration in the medullary zone of mesenteric lymph nodes. There was no significant difference reported for the treatment groups.

Mean±SD for macrophage infiltration of the medullary zone

vehicle	SR33589B 100 mg/kg/day	Diltiazem 100 mg/kg/day
3.6±0.6	3.6±0.6	3.6±0.5

The results for the cortical zones also showed no differences.

Mean±SD for macrophage infiltration of the cortical zone

vehicle	SR33589B 100 mg/kg/day	Diltiazem 100 mg/kg/day
2.3±1.2	2.4±1.1	2.8±1.1

The study was adequate and indicates that under the conditions of the study dronedarone had no detectable influence on permeability and functionality of vessels in mesenteric lymph nodes of female Sprague-Dawley rats. The study did not support the hypothesis of altered blood flow to the lymph nodes promoting carcinogenic changes.

Bacterial Reverse Mutation Test of Dronedarone Impurity on Salmonella Typhimurium (Ames test)

Study location: Sanofi-Synthelabo, Porcheville, France
Study number: FSRFU-HIS1692-EN-E01
Report Number: his1692
Study dates: experiment started July 20, 2005
GLP: statement included
QA: yes
Test article: SR 194090 (a dronedarone impurity), batch A-CRS-050018, purity 98.45%
Vehicle: DMSO
Note: either the vehicle was contaminated or there was column carryover.

Control of stock solutions	In the first and second tests for each dose level tested the stock solution in the DMSO was analyzed and found acceptable. In solvent control a presence of peak corresponding to retention time of SR194090 estimated at about 0.8 µg/mL to 0.2 µg/mL was noted. This does not jeopardize the scientific value of this study.

Concentrations tested:

Preliminary cytoxicity test (TA98 and TA100), plate incorporation test:
0, 50, 125, 250, 1250, 2500, 5000 µg/plate ±S9 mix

Mutagenicity test (TA98, TA100, TA102, TA1535, TA1537), plate incorporation and pre-incubation methods:
0, 50, 125, 250, 500, 1250, 2500, 5000 µg/plate ±S9 mix

Treatment duration was 48-72 hours.

S9 mix: prepared from the livers of male Sprague-Dawley rats treated with Aroclor 1254 (Moltox, Inc).

Positive controls:
Without S9: sodium azide (Na azide), 2-nitrofluorene, 9-aminoacridine, Mitomycin C
With S9: 2-aminoanthracene

Number of plates/concentration/test: 1(dose-range finding cytotoxicity test), 3(mutagenicity tests)
Number of independent mutagenicity tests: 2(one plate incorporation and one pre-Incubation) for all strains

In the dose-range finding study, precipitates were observed for both tester strains without S9 at concentrations ≥2500 µg/plate. Precipitation was reported for TA102 at concentrations ≥500 µg/plate both with and without S9 mix.

The sponsor provided criteria by which the acceptability of the assay was assessed and the criteria for a positive response.

Criteria for positive response:

- if, for at least one dose level, the induction factor is greater than 2 with the strains TA98, TA100 and TA102, and greater than 3 with the strains TA1535 and TA1537 (6).
- and the increase in the number of revertants is dose-related or observed at the highest dose level.
- and the values are out of the range of the historical negative control data.
- and the response is reproducible using the same experimental conditions.

4.6 Data acceptance criteria

- Mean number of revertants in negative controls has to be within the range of historical control values,
- Positive controls have to induce a marked increase in the number of revertant colonies for all strains, therefore confirming the sensitivity of the strains and the activity of S9-mix (AA),
- At least three non-cytotoxic dose levels.
- At least five analysable dose levels.

The positive controls produced appropriate responses. Under the conditions of the assay there were no positive responses to the test article in the bacterial strains tested. The presence of test article in the vehicle control cast some doubts about the adequacy of the study although the sponsor does not feel that the integrity of the data was compromised.

For the solvent control a presence of peak corresponding to retention time of SR194090 estimated at about 0.8 µg/mL to 0.2 µg/mL was noted. Possibly due to a technical failure, the origin of this presence still unclear but this does not jeopardize the scientific value of this study (see deviation in section (4.11)).

The range of historical controls was provided. Some of the present control results are slightly high compared to the mean values for the historical controls but are for the most part within the historical ranges.

In Vitro Gene Mutation Test of Dronedarone Impurity at the Locus TK in Mouse Lymphoma L5178Y Cells

Study location: Sanofi-Synthelabo, Porcheville, France
Study number: FSRFU-LYM0193-EN-E01
Report number: LYM0193
Study date: July 19, 2005
GLP: statement included
QA: yes
Test article: SR194090, an impurity of dronedarone, batch A-CRS-050018,
Vehicle: DMSO. Analysis did not reveal test article in the vehicle.

Concentrations tested (μg/ml)

Dose-range finding cytotoxicity test	
-S9 (3 hour exposure)	5-10-25-50-75-100-150
+S9 (3 hour exposure)	5-10-25-50-75-100-150
-S9 (24 hour exposure)	0.01, 0.05, 0.1, 0.5, 1, 2.5, 5
First test	
-S9 (3 hour exposure)	5, 10, 12.5, 15, 17.5, 20
+S9 (3 hour exposure)	25, 50, 55, 60, 65, 70
Second test	
-S9 (24 hour exposure)	0.5, 1, 2, 2.5, 3, 4
Complementary assays	
-S9 (3 hour exposure)	1, 2.5, 5, 7.5, 10, 12.5, 15
+S9 (3 hour exposure)	25, 30, 40, 50, 55, 60, 65, 70, 75, 80, 85

Positive controls: -S9 methylmethane sulfonate (MMS)
+S9 Cyclophosphamide (CP)

Number of cultures/concentrations 1: dose-range finding cytotoxicity test
2: mutagenicity tests

Number of independent assays: -S9 (3 hour exposure) : 2
+S9 (3-hour exposure) : 2
-S9 (24 hour exposure) : 1

Precipitation was seen at concentrations ≥150 μg/ml.

There were blips in the mutation frequency at the concentrations where relative total growth was decreased to appropriate levels. However, dose-responses were not apparent and significant increases were not apparent except under one set of conditions (second assay of 24 hours, -S9, shown below). There were several small increases in small colony count. The lack of consistent signals is suggestive that the findings are random occurrences rather than indications of genotoxicity. The results are shown below.

LYM0193 First test in the absence of S9-mix (3-hour exposure)

Compound		Day 0 factor	CE0 (%)	S (%)	RS (%)	SG	CE2 (%)	RTG (%)	Adj RTG (%)	MF ($\times 10^6$)	Stat.	MI	MF small colonies ($\times 10^6$)	MF large colonies ($\times 10^6$)
solvent control	A	0.95	104.62	99.28	100.46	12.46	87.96	92		103.64		1.07	24.19	75.91
	B	1.05	93.52	98.30	99.47	11.31	116.02	110		89.49		0.92	37.50	47.41
	Totals	1.00	98.82	98.82	100.00	11.89	100.43	100	100	97.04		1.00	32.13	60.59
SR194090 5 µg/mL	A	1.06	90.68	95.71	96.85	10.15	84.09	71		55.12		0.57	25.31	28.55
	B	1.13	70.65	80.16	81.12	9.12	86.64	66		34.05		0.35	12.15	21.43
	Totals	1.10	79.86	87.46	88.50	9.63	85.35	69	76	44.35	NS	0.46	18.60	24.93
SR194090 10 µg/mL	A	0.94	45.98	43.38	43.90	6.45	94.99	51		70.29		0.72	36.90	33.97
	B	1.02	46.66	47.77	48.34	5.97	81.64	41		89.11		0.92	46.37	39.53
	Totals	0.98	46.32	45.56	46.10	6.21	87.96	46	45	79.30	NS	0.82	41.44	36.69
SR194090 12.5 µg/mL	A	0.82	15.85	12.95	13.10	2.02	90.68	15		162.47		1.67	93.68	60.66
	B	0.85	15.01	12.73	12.88	1.76	104.62	15		134.18		1.38	75.33	49.80
	Totals	0.83	15.43	12.84	12.99	1.89	97.26	15	12	147.89	NS	1.52	84.18	55.06
SR194090 15 µg/mL	A	0.67	3.00	2.00	2.02	0.32								
	B	0.57	6.51	3.72	3.77	0.40								
	Totals	0.62	4.73	2.93	2.96	0.36	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
SR194090 17.5 µg/mL	A	0.43	0.00	0.00	0.00	0.20								
	B	0.56	0.00	0.00	0.00	0.28								
	Totals	0.50	0.00	0.00	0.00	0.24	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
SR194090 20 µg/mL	A	0.32	0.00	0.00	0.00	0.21								
	B	0.29	0.33	0.10	0.10	0.32								
	Totals	0.31	0.16	0.05	0.05	0.27	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
MMS 25 µg/mL	A	1.03	47.36	48.58	49.15	6.87	32.58	19		610.30		6.29	318.65	232.57
	B	1.02	55.50	56.57	57.24	7.31	27.33	17		969.97		10.00	379.93	488.66
	Totals	1.02	51.30	52.45	53.07	7.09	29.90	18	18	772.13		7.96	347.24	347.24

LYM0193 Second test in the absence of S9-mix (24-hour exposure)

Compound		Day 0 factor	CE0 (%)	S (%)	RS (%)	SG	CE2 (%)	RTG (%)	Adj RTG (%)	MF (x10⁻⁶)	Stat.	MI	MF Small colonies (x10⁻⁶)	MF Large colonies (x10⁻⁶)
solvent control	A	0.91	98.04	89.50	90.57	14.63	70.65	96		142.43		1.05	61.58	73.75
	B	1.09	99.62	108.30	109.58	13.49	82.85	104		129.18		0.95	69.90	52.51
	Totals	1.00	98.82	98.82	100.00	14.06	76.45	100	100	135.79		1.00	66.26	62.50
SR194090 0.5 µg/mL	A	0.82	92.08	75.55	76.45	12.54	89.30	104		149.53		1.10	91.68	48.72
	B	0.88	84.09	74.18	75.06	12.32	79.28	91		185.82		1.37	107.15	65.72
	Totals	0.85	87.96	74.88	75.77	12.43	84.09	97	82	166.94	NS	1.23	99.19	56.83
SR194090 1 µg/mL	A	0.74	73.75	54.87	55.52	11.82	84.09	92		158.79		1.17	108.41	41.69
	B	0.86	63.06	54.46	55.11	10.31	75.90	73		180.42		1.33	99.83	68.64
	Totals	0.80	68.18	54.80	55.45	11.06	79.86	82	66	169.33	NS	1.25	104.44	54.48
SR194090 2 µg/mL	A	0.57	52.42	29.88	30.24	7.17	72.70	49		151.69		1.12	100.08	44.39
	B	0.60	42.67	25.54	25.84	5.95	67.69	37		228.04		1.68	148.65	64.27
	Totals	0.58	47.36	27.67	28.00	6.56	70.15	43	25	187.94	NS	1.38	123.31	53.97
SR194090 2.5 µg/mL	A	0.36	27.33	9.81	9.93	4.01	81.64	30		234.13		1.72	139.06	74.53
	B	0.33	26.82	8.90	9.01	4.75	87.96	39		262.45		1.93	175.50	62.53
	Totals	0.35	27.07	9.35	9.46	4.38	84.72	35	12	248.59	*	1.83	157.61	68.36
SR194090 3 µg/mL	A	0.36	12.18	4.33	4.38	2.75	75.90	19						
	B	0.33	10.23	3.40	3.44	2.52	68.66	16						
	Totals	0.34	11.20	3.85	3.90	2.63	72.18	18	6	(1)	(1)	(1)	(1)	(1)
SR194090 4 µg/mL	A	0.19	0.98	0.18	0.18	0.95								
	B	0.27	0.33	0.09	0.09	0.76								
	Totals	0.23	0.65	0.15	0.15	0.86	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
MMS 5 µg/mL	A	0.89	65.80	58.70	59.40	14.46	60.44	81		703.82		5.18	335.43	226.58
	B	0.87	66.74	57.97	58.66	13.38	57.11	71		788.70		5.81	361.88	257.99
	Totals	0.88	66.27	58.34	59.03	13.92	58.75	76	67	745.07		5.49	348.40	241.88

LYM0193 Complementary assay to the first test in the absence of S9-mix (3-hour exposure)

Compound		Day 0 factor	CE0 (%)	S (%)	RS (%)	SG	CE2 (%)	RTG (%)	Adj RTG ($\times 10^{-6}$)	MF ($\times 10^{-6}$)	Stat.	MI	MF small colonies ($\times 10^{-6}$)	MF large colonies ($\times 10^{-6}$)
solvent control	A	0.96	77.01	73.94	93.94	9.23	87.96	98		86.15		0.67	46.24	36.69
	B	1.04	80.45	83.66	106.29	11.16	74.82	101		178.48		1.39	81.33	85.27
	Totals	1.00	78.71	78.71	100.00	10.19	81.04	100	100	128.11		1.00	62.51	58.96
SR194090 1 µg/mL	A	0.79	53.94	42.79	54.36	9.84	81.64	97		100.28		0.78	46.37	53.29
	B	1.08	58.75	63.27	80.38	10.21	94.99	117		92.69		0.72	45.80	45.80
	Totals	0.94	56.30	52.64	66.88	10.03	87.96	107	101	96.58	NS	0.75	46.24	49.46
SR194090 2.5 µg/mL	A	1.02	69.65	70.94	90.13	9.00	71.66	78		172.23		1.34	76.75	84.91
	B	0.91	71.66	64.88	82.43	9.54	72.70	84		134.05		1.05	75.66	52.07
	Totals	0.96	70.65	67.96	86.34	9.27	72.18	81	78	152.78	NS	1.19	76.20	68.19
SR194090 5 µg/mL	A	0.86	66.74	57.21	72.69	8.33	68.66	69		123.72		0.97	75.88	42.96
	B	1.00	44.64	44.80	56.92	7.89	68.66	66		155.88		1.22	84.35	67.51
	Totals	0.93	54.72	50.91	64.68	8.11	68.66	67	62	139.62	NS	1.09	80.10	55.13
SR194090 7.5 µg/mL	A	0.94	53.17	50.12	63.68	7.58	77.01	71		118.38		0.92	63.91	49.16
	B	0.86	49.47	42.73	54.29	7.91	68.66	66		170.12		1.33	105.96	55.13
	Totals	0.90	51.30	46.33	58.86	7.75	72.70	68	61	142.81	NS	1.11	83.70	52.07
SR194090 10 µg/mL	A	0.74	32.04	23.81	30.25	4.25	71.66	37		172.23		1.34	80.82	80.82
	B	0.81	30.43	24.73	31.42	3.78	71.66	33		153.87		1.20	101.52	48.92
	Totals	0.78	31.23	24.29	30.87	4.01	71.66	35	27	162.99	NS	1.27	91.09	64.68
SR194090 12.5 µg/mL	A	0.63	12.18	7.71	9.80	1.78	63.96	14						
	B	0.53	12.18	6.43	8.18	1.60	75.90	15						
	Totals	0.58	12.18	7.07	8.99	1.69	69.65	14	8	(1)	(1)	(1)	(1)	(1)
MMS 25 µg/mL	A	0.80	43.98	35.05	44.53	7.76	18.42	17		1122.16		8.76	743.63	314.49
	B	0.94	35.38	33.28	42.29	6.63	19.74	16		1107.39		8.64	591.71	461.79
	Totals	0.87	39.53	34.34	43.64	7.19	19.07	17	15	1114.54		8.70	664.62	389.32

Tissue Distribution of Total Radioactivity and Histopathology in Lymph Nodes Following Single and/or 28 days Repeated Oral Administration of[^{14}C]-SR33589B or SR33589B at 25 mg/kg/day and 100 mg/kg/day to Male Sprague Dawley rats. Amended Final Study Report

Study location: Sanofi-Aventis Recherche and Developpement, Longjumeau, France
Study number: FSRFU-RDI0020-EN-E02
Report number: RDI0020
Study dates: initiated May 29, 2006
GLP: no
QA: no
Test article:

[^{14}C]-SR33589B batches SYN1763 (25 mg/kg/day), 44.4 kBq/mg (1.2 µCi/mg)
And SYN1795 (100 mg/kg/day), 11.1 kBq/mg, (0.3 µCi/mg)
SR33589B batch CL-05754
Salt/base ratio : 1.07

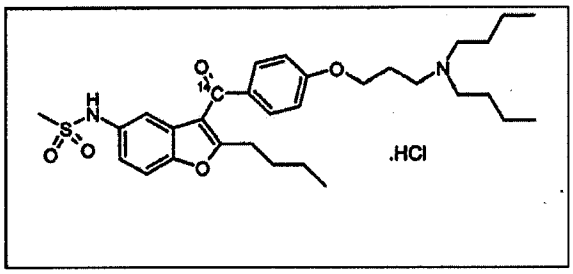

Figure (2.2.1) 1 - **Chemical structure of SSR33589B** and position of ^{14}C-labeling

Vehicle: 0.6% methylcellulose in distilled water
Animals: male Sprague-Dawley rats (OFA SD (IOPS caw))
Animals were fasted overnight prior to administration day 1 and day 28.
Treatment duration: single (once on day 1) or repeated doses (daily for 28 days)

The stated purpose of this study was to provide information on the tissue distribution and accumulation of total radioactivity in mesenteric and mandibular lymph nodes by autoradiography after single or repeated oral dosing of [^{14}C]-SR33589B (25 and 100 mg/kg/day).

Sponsor's summary of study design for tissue distribution

Substudy	1 (Tissue distribution)							
Sex	Male (M)							
Route	Oral							
Group	1				2			
Dose (mg/kg/day)	25				100			
(MBq/kg/day)	1.11 (30 µCi/kg/day)							
Volume (mL/kg/day)	5							
Sacrifice times after dosing (h)	Day 1		Day 28		Day 1		Day 28	
	4	24	4	24	4	24	4	24
Number of animals	3 rats/time/dose (24 rats)							
Animal numbers	1 2 3	4 5 6	7 8 9	10 11 12	111 112 113	114 115 116	117 116 119	120 121 122

At each time point 3 rats were euthanized.

- Blood samples were collected at time of euthanasia.
- 2 animals were used for quantitative image analysis.
- 1 animal was used for autohistoradiography
- Mesenteric and tracheobronchial lymph nodes were removed and fixed in 4% buffered formalin: these samples were sent to Sanofi-Aventis in Montpellier for autohistoradiography

Sponsor's summary of study design for microscopy

Substudy	2 (microscopic examination)		
Sex	Male (M)		
Route	Oral		
Group	3	4	5
Dose (mg/kg/day)	0 (vehicle)	25	100
Volume (mL/kg/day)	5		
Sacrifice	Day 29		
Number of animals	10 rats/dose (30 rats)		
Animal numbers	131 to 140	141 to 150	151 to 160

Rq: Day 1 is the first day of administration

At necropsy, mesenteric and tracheo-bronchial lymph nodes were collected for light microscopy and transmission electron microscopy (TEM).

Total radioactivity was determined by combustion and liquid scintillation counting (LSC). Other aliquots were analyzed by direct LSC. Liver samples also were collected but it is not clear which animals they were derived from. The methodology section is badly organized and I am not clear what procedures were performed on which animals. At the very least, total radioactivity was determined and used as a standards of calibration for quantification purposes.

The sponsor summarized the parameters analyzed:

2.9 Evaluation criteria

- Blood and plasma radioactivity concentrations after single or repeated doses
- Distribution and accumulation of TR in mesenteric and mandibular lymph nodes at day 1 and day 28
- Lymph nodes/blood concentration ratios
- Mesenteric/mandibular lymph nodes concentration ratios
- Accumulation index (Rac.) for blood, plasma, liver and lymph nodes
- Microscopic observations of mesenteric and tracheo-bronchial lymph nodes

2.10 Expression of results

- Total radioactivity concentrations in blood, plasma, liver, mesenteric and mandibular lymph nodes are expressed as ngEq./g.
- Qualitative tissue distribution data are presented as illustrations.
- Macroscopic and histomorphological observations are reported using PathData software, version 6.2b5 (PathData ® System). Results included incidence tables of macroscopic and microscopic examination and individual animal listing of observations.

It was not explained when the radiolabeled drug was administered or when the unlabeled drug was administered.

Results

Unscheduled mortality was reported for 5 animals. The sponsor decided that the deaths of the 2 HD animals might be related to treatment.

During animal experiment, 5 animals died without obvious explanation:
- Rat 7, Group 1, ^{14}C-SR35589B, 25 mg/kg/day (died on D17),
- Rat 142, Group 4, SR35589B, 25 mg/kg/day (died on D26),
- Rat 149, Group 4, SR35589B, 25 mg/kg/day (died on D25),
- Rat 156, Group 5, SR35589B, 100 mg/kg/day (died on D27),
- Rat 158, Group 5, SR35589B, 100 mg/kg/day (died on D16).

No rat was used for autohistoradiography.

Tissue concentration ratios

		Dose (mg/kg/day)			
		25		100	
Concentration ratio		4h	24h	4h	24h
Mesenteric ln / Blood	day 1	7.3	≥ 5.2	32.7	NC
	day 28	38.4	≥ 13.3	15.8	≥ 21.2
Mandibular ln / Blood	day 1	4.6	NC	4.2	NC
	day 28	6.2	NC	5.1	≥ 8.8
Mesenteric ln / Mandibular ln	day 1	1.6	≥ 1.2	7.7	NC
	day 28	6.2	≥ 3.0	3.1	2.4

(ln): lymph nodes - (≥): for concentration/BLQ ratio
(NC): not calculable

Accumulation index

	Rac. (D28/D1)			
	25 mg/kg/day		100 mg/kg/day	
Body fluids / tissues	4h	24h	4h	24h
Blood	0.6	NC	1.1	NC
Plasma	0.6	NC	1.2	≥ 1.6
Liver	0.7	4.0	1.4	≥ 3.5
Mesenteric ln	3.3	2.4	0.6	≥ 4.5
Mandibular ln	0.8	NC	1.4	≥ 1.9

(Rac.): accumulation index (conc. at day 28 / conc. at day 1)
(ln): lymph nodes - (≥): for concentration/BLQ ratio
(NC): not calculable

Dose-proportionality

	day 1		day 28	
	4h	24h	4h	24h
Body fluids / tissues	$Conc._{100mg}$ / $Conc._{25mg}$ ratio			
Blood	3.6	NC	6.6	NC
Plasma	3.1	NC	5.9	NC
Liver	3.1	NC	6.1	2.6
Mesenteric ln	16.3	NC	2.7	6.1
Mandibular ln	3.3	NC	5.4	NC

(ln): lymph nodes - (NC): not calculable

After single and repeated oral administration of [^{14}C]-SR33598B or repeated oral administration of SR33598B, it was noted that greater levels of radioactivity were located in the mesenteric lymph nodes than the mandibular lymph nodes. There was some accumulation of radioactivity in the mesenteric lymph nodes and little to no apparent accumulation in the mandibular lymph nodes.

Receptor Binding of Dronedarone and the Metabolites SR35021A and SR90154

I requested, by email, information regarding the receptor binding of the parent drug and 2 major metabolites. The sponsor referred me to the following studies, which I have assembled into a table along with the review status and content :

Study	status	content
685-4-001	Submitted in NDA 21913	Effect of dronedarone on enzymes of cellular function (adenylate cyclase, 5'-nucelotidase, several ATPases)
685-4-023	Submitted in NDA 21913	Effect of dronedarone on β and α adrenergic receptors, Ca channels, Na+/Ca++ and Na+/H+ exchangers.
685-4-043	Submitted and reviewed in NDA 21913	Dronedarone was tested against β-adrenergic receptors, and adenylate cyclase activity
685-4-052	Submitted and reviewed in NDA 21913	SR35021A, N-debutyl metabolite, inhibited binding to the L-type calcium channel and the cardiac Na+ channel with IC50 values from 1 to 7 μM. A very limited selection of receptors was examined.
685-4-067	Submitted and reviewed in NDA 21913	SR90154, the O-propionic acid metabolite had modest effects of 10% to 38% inhibition at 30 μM for the α1 adrenergic receptor, muscarinic cholinergic, A1 purinergic, L-type calcium channel (brain), Na+ channel and $K+_{ATP}$ channels. A very limited selection of receptors was examined.
CTT-0168	Submitted and reviewed in NDA 21913	Dronedarone showed binding to cardiac β adrenergic (only receptor type tested) receptors, IC50 =1755±360 nM
Van Beeren et al 2003	New material	Publication examining effects of dronedarone and SR35021A On T3 binding to TRα1 and TRβ1 in vitro. In vivo study examining TSH, rT3, T3 and T4 in male Wistar rats.

The Van Beeren publication showed that, *in vitro*, dronedarone produced a slight but statistically significant ($p<0.01$) 14±3% inhibition of of T3 binding to both TRα1 and TRβ1 receptors but no inhibition of binding of T3 to TRβ1. The debutylated dronedarone at 100 μM strongly inhibited binding of T3 to TRα1 by 77±3% ($p<0.01$) and inhibited binding to TRβ1 by 25±4%($P<0.01$) compared with control. The concentration required for an observable effect indicates low potency.

The *in vivo* studies used male Wistar rats given either water (controls), an aqueous suspension of 50 mg/kg or 100 mg/kg dronedarone given by oral gavage daily for 2 weeks. Amiodarone 100 mg/kg was used as a comparator. At the end of the dosing period, blood was collected for analysis of plasma cholesterol, triglycerides, T4, T3, rT3 and TSH. Livers were collected for analysis of LDL-cholesterol receptor (LDL-r) expression and type 1 deiodinase (D1) activity. The author's summary of these results is shown below.

TABLE 3. Effect of Dron or AM treatment in rats on ΔBW, plasma thyroid hormones, and plasma lipids

	Controls (n = 8)	Dron50 (n = 8)	Dron100 (n = 6)	AM100 (n = 8)
Δ BW (g)	87 (63–93)	70 (50–90)[a]	65 (−25–75)[b]	62 (51–75)[b]
TSH (ng/ml)	1.02 ± 0.24	1.05 ± 0.54	0.87 ± 0.36[c]	1.87 ± 0.88[b]
T_4 (nmol/liter)	74 ± 9.6	62 ± 13	57 ± 12[d]	72 ± 15
FT_4 index	115 ± 15	97 ± 20	89 ± 18[d]	113 ± 23
FT_4 (pmol/liter)	39.5 ± 7.3	33.4 ± 7.1	33.7 ± 8.1	40.8 ± 9.0
T_3 (nmol/liter)	1.38 ± 0.10	1.17 ± 0.16[d]	1.09 ± 0.19[b]	1.03 ± 0.17[b]
FT_3 index	2.21 ± 0.16	1.84 ± 0.25[b]	1.71 ± 0.30[b]	1.61 ± 0.26[b]
FT_3 (nmol/liter)	0.10 ± 0.01	0.09 ± 0.02	0.10 ± 0.01	0.15 ± 0.02[b]
Total cholesterol (mmol/liter)	2.05 ± 0.18	1.86 ± 0.25	1.81 ± 0.50	2.27 ± 0.29[b]
LDL-chol (mmol/liter)	0.93 ± 0.05	0.95 ± 0.13	0.86 ± 0.37	1.15 ± 0.42[c]
HDL-chol (mmol/liter)	0.96 ± 0.10	0.85 ± 0.18	1.05 ± 0.11	1.20 ± 0.29[a]
Heart rate (beats/min)	382 ± 35	365 ± 26	390 ± 26	351 ± 23[a]
QTc interval (msec)	0.114 ± 0.013	0.125 ± 0.009[f]	0.142 ± 0.023[a]	0.141 ± 0.018[a]

Values are the mean ± SD. Δ BW, The difference between d 1 and d 15 body weights (median and range).
[a] $P < 0.05$ *vs.* controls.
[b] $P < 0.01$ *vs.* controls.
[c] $P = 0.1$ *vs.* controls.
[d] $P < 0.02$ *vs.* controls.
[e] $P = 0.07$ *vs.* controls.
[f] $P = 0.055$ *vs.* controls.

The author notes that:

> The heart has an abundance of TR[thyroid receptor]α1 relative to TRβ1, whereas tissues such as the liver express TRβ1 more abundantly than TRα1...One of the side-effects of amiodarone [AM] is an increase in plasma cholesterol caused by a down-regulation of the T3-dependent LDL-r [low density lipoprotein receptor] gene in the liver ...
>
> Because of the higher affinity of DBDron [debutylated dronedarone] to TRα1, we hypothesized that the effect of Dron on heart rate and QTc interval would be similar to that of AM but that Dron, in contrast to Amiodarone, will not cause hypercholesterolemia and changes in liver LDL-r expression, as T3-induced changes in cholesterol metabolism are mainly mediated via TRβ1.

The above table of data supports the author's hypothesis. The author concludes the paper with the statement:

> In conclusion, the *in vitro* and *in vivo* data presented in this paper indicate that Dron, via its metabolite DBDron, is a TRα_1-selective inhibitor of T_3 binding to its receptor. This isoform selectivity can explain the effects of Dron on the heart (a mainly TRα_1 organ) and the lack of effect on the liver (a mainly TRβ_1 organ) and may also point the way to designing TR isoform-specific antagonists.

While this provides some insight into the receptor binding and thyroid effects, it still isn't the basic receptor binding screen that is typically a part of new drug characterization.

Overall Summary and Evaluation

Dronedarone is a benzofuran derivative structurally similar to amiodarone and demonstrating electrophysiologic characteristics of all 4 Vaughan-Williams classes of anti-arrhythmic compounds. Dronedarone was originally presented in NDA 21913 for treatment of atrial fibrillation (AF) or atrial flutter (AFL).

The Division issued a "non-approvable" letter for NDA 21913, for both clinical and non-clinical reasons. The clinical reasons for non-approval included an unfavorable risk: benefit relationship. The potential symptomatic improvement offered by the drug was also weighed against the nonclinical issues of carcinogenicity, teratogenicity, and endocrine effects (thyroid gland and female cyclicity). The complete response to these concerns was filed under NDA 22425 with a new indication of treating patients with AF/AFL or with a history of such events to reduce the risk of cardiovascular hospitalization or death.

Carcinogenicity

The sponsor provided measurement of circulating prolactin levels after a single dose and after 28 days repeated dosing of dronedarone. The reports do not provide details of what, if anything, was done to control the variability from diurnal and circadian factors. Accepting the data at face value, there were statistically significant increases in mean prolactin levels after 1 dose and after 28 days of repeated dosing. The duration of these elevations in mean prolactin levels has not been determined. Increased prolactin via dopamine 2 receptor antagonism is a generally accepted mechanism for mammary tumors in rodents.

In the Non-Clinical Summary, the following information was provided:

Table 14 - Binding assays carried out by MDS Panlabs (February 12 1999)

	Ligand	DRONEDARONE IC_{50}
Dopamine D_1	[^{3}H]SCH23390	>10 µM (30%)
Dopamine D_{2L}	[^{3}H]Spiperone	>10 µM (33%)
Dopamine D_3	[^{3}H]Spiperone	1.1 µM
Dopamine $D_{4.2}$	[^{3}H]Spiperone	4.2 µM

Material source: human recombinant, mammalian

There is weak binding to the dopaminergic receptors, in particular the D2 isoform. It is not known if the effects were agonistic or antagonistic. There was no comparison to the receptor binding affinities of drugs demonstrated to cause mammary tumors through prolactin increases.

As noted in the original NDA review, circulating prolactin levels as originally measured decreased or showed minimal changes that could have been within the realm of normal variability. The increases reported in the studies described in the current submission were relatively small compared to those reported for D2 receptor agonists such as haloperidol and clozapine (Xu et al., 2002). Increased prolactin levels are also correlated with irregularities of estrus cycles, something noted in other toxicology studies for this NDA and which the sponsor could have included in their case for a prolactin mechanism.

There are several points contrary to the sponsor's position that 1) the mammary tumors are due to increased prolactin and 2) the prolactin mechanism is irrelevant to humans:

1. It is not agreed that an increase in serum prolactin in rodents is irrelevant to humans.
2. It has not been demonstrated that increased serum prolactin does not occur in humans treated with this drug.
3. The sponsor has demonstrated minimal binding of the drug to the D2 receptor and has not demonstrated any binding of a metabolite to the relevant receptor.
4. The sponsor has not explored any other potential mechanism of carcinogenesis.

An alternative carcinogenic mechanism is an estrogenic effect, to which mice are believed to be more sensitive than rats. An estrogenic mechanism would also account for the hemangiomas and hemangiosarcomas due to the role that estrogen plays in vascular development in both species. No data was presented exploring this possible mechanism, including receptor binding profiles.

Below is a summary table of tumors listed for drugs demonstrated to increase circulating prolactin in rodents. All of these drugs were associated with an increased incidence of mammary tumors in mice and/or rats. Olanzapine and Quetiapine appear to be unusual in the list in that Olanzapine had no tumors reported in rats and neither drug appeared to cause pituitary tumors in mice. There were however, hemangiomas and hemangiosarcomas, similar to dronedarone. The remainder of the drugs accepted to cause rodent tumors by a prolactin mechanism show combinations of pituitary, mammary and pancreas tumors in one or both species. Dronedarone does not fall into that apparent pattern.

Summary of Tumors Reported in Package Inserts for Anti-Psychotics

drug	rats				mice			
	Pituitary	Mammary	Pancreas	other	Pituitary	Mammary	Pancreas	other
Aripiprazole(Abilify)		x		adrenal	x	x		
Paliperdone (Invega)	x	x	x		x	x	x	
Ziprasidone (Geodon)					x	x		
Quetiapine(Seroquel)		x						
Risperidone (Risperdal)		x(m+f)	x(m)		x	x		
Olanzapine(Zyprexa)						x		Hemangiomas and hemangiosarcomas*
Clozapine(clozaril)	No findings reported							
Zuclopenthixol(Clopixol)		x	x					

* did not occur in second study

The sponsor proposed that the increased incidence of histiocytic sarcoma, angiomatous hyperplasia, hemangioma and hemangiosarcoma may have been caused by changes in local blood flow, secondary to accumulation of foamy macrophages. The histologic changes of sinusal ectasia, sinusoidal erythrocytes, erythrophagocytosis and hilar angiectasis in the mesenteric lymph nodes in the rat carcinogenicity study with dronedarone were presented as indicative of chronic local vasodilation. At the time of the original report these findings plus angiomatous hyperplasia and the hemangiomas were described by the sponsor as part of a continuum of tumoral transformation. The sponsor presented a radiolabel distribution study and a study to demonstrate altered blood flow to the mesenteric lymph nodes. Whether or not chronic vasodilation is an accepted mechanism of carcinogenesis, neither study supported the hypothesis of altered blood flow. It was reported that the tumor incidences seen in the dronedarone studies were within the background range of the NTP studies reported in Haseman et al. However, the referenced publication is Haseman et al. "Use of dual control groups to estimate false positive rates in laboratory animal carcinogenicity studies." Fundamental and applied Toxicology: 573-584 (1986). There is a question of the relevance of the Charles River SD rats and CD-1 mice used in the historical studies to the SD(IOPS Caw) rats and the CD-1 mice used almost 20 years later.

Historical control data from in-house studies are limited to a single study:

Table 11 – Historical control rates of histiocytic sarcoma from in-house (same site) CD-1 female mice - 24-month oncogenicity study

	Male mice		Female mice	
Number of animals	**120**		**120**	
	No. of tumors	Incidence (%)	No. of tumors	Incidence (%)
Histiocytic sarcoma	4	3.3	9	7.5

Reading the reference provided by the sponsor, I could not locate the source of the numbers presented below:

Table 12 – Incidence of histiocytic sarcoma in untreated control CD-1 mice for 18 color studies - combined summary of all studies

	Male mice			Female mice		
Number of groups	**37**			**37**		
Number of animals	**2221**			**2212**		
	No. of tumors	Incidence (%)	Range (%)	No. of tumors	Incidence (%)	Range (%)
Histiocytic sarcoma	56	2.5	0-13.33	134	6.1	0-16.7

Going to the Charles River Website, the reference "Spontaneous Neoplastic Lesions in the Crl: CD-1(ICR) Mouse in Control Groups from 18 months to 2 year Studies" March, 2005, provided the following control data from studies conducted from 1987-2000:

Summary of selected neoplasias seen in control animals from the Charles River database

	Crl:CD1(ICR)		Crl:CD®(SD)	
	Male mice	Female mice	Male rats	Female rats
	Range %	Range %	Range %	Range %
Histiocytic sarcoma	1.11-8.00	1.67-18.33	0.77-6.00	1.11- 3.08
Mammary adenocarcinomas		0.78-8.33	0.56-4.29	8.57-58.33
hemangiomas	Not listed for whole body/mult. organ	1.43-2.67	0.56-3.33	Not listed for whole body/mult organ
hemangiosarcomas	1.67-12.00	1.67-12.00	0.56-2.67	1.43-1.67

The tumor incidences in CD1 mice used in the dronedarone carcinogenicity studies were within the historical ranges reported in the submission for histiocytic sarcomas and hemangiosarcomas and within the ranges that I found on the Charles River website. In the original reports, however, the sponsor listed historical control incidences of hemangiosarcoma from the published literature as 0-1.9%. There were no reports of hemangiosarcoma in 720 mice of the same strain used in another study in the sponsor's facilities.

Incidence of histiocytic sarcomas in mice: NDA 21913 carcinogenicity studies

	Males				females			
Dose mg/kg	0	75	150	300	0	75	150	300
# examined	120+	60	60	60	120+	60	60	60
Histiocytic sarcoma	1	2	2	5**	7	4	2	7
%	0.83	3.3	3.3	8.3	5.8	6.7	3.3	11.7

+pooled samples **p=0.004

Incidence of hemangioma and hemangiosarcoma in mesenteric lymph nodes in mice: NDA 21913 carcinogenicity studies

	Males				females			
Dose mg/kg	0	75	150	300	0	75	150	300
# examined	116+	59	60	58	117+	58	59	60
hemangioma	-	1	-	-	-	-	-	2*
%	-	1.7	-	-	-	-	-	3.3
Hemangiosarcoma	-	-	-	-	-	-	-	2*
%								3.3

+pooled controls **p=0.032 (mortality adjusted incidences)

It is not clear why the sponsor chose to assess structural indicators of vascularity instead of a more dynamic measure of blood flow. The analysis of the tissue sections was very limited, raising a question as to how well the entire tissue was represented.

In the sponsor's textual discussion of the irrelevance of the carcinogenicity findings, they say that "Hemangiomas are not precancerous changes and do not transform into malignant hemangiosarcomas in either animals or man." Then why does the NTP recommend combining these two tumor types when doing statistical analysis (McConnell, et al.)?

There were three different tumor types found in the carcinogenicity studies. Hemangiomas appeared in both rodent species. Hemangioma and hemangiosarcoma, were seen in both sexes of mice. The sponsor postulates 3 different mechanisms of carcinogenicity occurring simultaneously to explain these tumors and the mammary tumors. Overall, the sponsor has not provided sufficient information to show that the carcinogenicity is not clinically relevant.

Endocrine Effects and Receptor Binding

The publication provided in response to my request for receptor binding data indicates some modulation of the thyroid axis by parent drug and one of the major metabolites (Van Beeren et al. 2003, Endocrinology 144(2):552-558 "Dronedarone acts as a selective inhibitor of 3,5,3'-triiodothyronine binding to thyroid hormone receptor-α1: in vitro and in vivo evidence."). However, the receptor profile that is usually a basic part of new drug characterization is still lacking.

The possible thyroid and cyclicity effects are reported to have been addressed clinically. As such, the material will be evaluated by the medical officer.

The non-clinical material presented by the sponsor does not completely address the Division's concerns.

References

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EE McConnell, HA Solleveld, JA Swenberg, GA Boorman. "Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. " JNCI 1986;76:283-289.

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/s/

Elizabeth Hausner
12/10/2008 08:28:10 AM
PHARMACOLOGIST
Elizabeth Hausner

Charles Resnick
12/10/2008 08:47:38 AM
PHARMACOLOGIST

MEMORANDUM
April 4, 2006

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-913

I concur with the recommendation by the pharmacology/toxicology reviewer, Dr. Elizabeth Hausner that the marketing application for Dronedarone (Multac®) is approvable pending resolution of several issues. Although some of these issues may be resolved post-marketing, others involve changes in the product label. In particular, I agree with Dr. Hausner that the labeling with respect to carcinogenicity and embryo-fetal findings is inadequate and should be resolved before approval. Dr. Hausner has also recommended that phototoxicity findings in guinea pigs should be incorporated into the product label. Although this is a decision to be made by the review division, my recommendation is that the issue of phototoxicity should be included in the product label only of there were clinical findings.

_____
Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director
Office of New Drugs

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/s/

Kenneth Hastings
4/4/2006 02:41:16 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-**913**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	June 10, 2005
PRODUCT:	dronedarone
INTENDED CLINICAL POPULATION:	**patients with atrial fibrillation or atrial flutter**
SPONSOR:	Sanofi
DOCUMENTS REVIEWED:	**Electronic submission.**
REVIEW DIVISION:	**Division of Cardio-Renal Drug Products**
PHARM/TOX REVIEWER:	**Elizabeth Hausner, D.V.M.**
PHARM/TOX SUPERVISOR:	**Al DeFelice, Ph.D.**
DIVISION DIRECTOR:	**Norman Stockbridge, M.D., Ph.D.**
PROJECT MANAGER:	**Russell Fortney**

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

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Approvable pending resolution of several issues

B. Recommendation for nonclinical studies: 1) qualification of impurity SR194090 1)Studies to elucidate the effect upon female cyclicity 2) receptor binding studies for the parent drug and main metabolites 3)generation of data to show the relevance or lack of relevance of the carcinogenic effects for humans

C. Recommendations on labeling

"Mechanism of Action" – "The drug has been shown non-clinically to have properties of the 4 Vaughan-Williams Classes. "Whether this statement stays in the labeling depends upon the clinical demonstration of these properties.

"Pharmacodynamic Properties"- The statement
"In animal models, dronedarone reduces the heart rate. It prolongs Wenckebach cycle length and AH-, PQ-, QT- intervals; with no marked effect on QTc-, HV- and QRS-intervals" should be removed as there is human data for these parameters.

"Dronedarone slightly decreases arterial blood pressure and myocardial contractility (dP/dt max) with no change in left ventricular ejection fraction and reduces myocardial oxygen consumption " should also be removed unless this is information derived from human studies.

The statement "Dronedarone has vasodilatory properties, more pronounced in coronary arteries (related to the activation of nitric oxide pathway) than in peripheral arteries " should be removed. This information is based on studies 1)in isolated organ culture 2) studies where dronedarone was intravenously infused into anesthetized dogs and 3)an intravenous infusion study using conscious instrumented dogs. The effects were transient and inconsistent at best.

"Carcinogenicity, Mutagenicity, Impairment of Fertility"
The statement "In animals, there was no increased incidence of tumors considered relevant for humans" should be removed. The statement should be replaced with "Dronedarone caused histiocytic sarcomas in male mice (300 mg/kg/day or 4.8X MRHD based on AUC comparisons) mammary adenocarcinomas in female mice (300 mg/kg/day or 8.2X MRHD based on AUC comparisons) and hemangiomas in male rats (70 mg/kg/day or 4.6X MRHD based on AUC comparisons). "

The statement "Dronedarone was not shown to alter fertility in animal studies up to 100 mg/kg/day" should be removed and replaced with " In studies conducted with female rats, Dronedarone caused an increase in irregular cycles ($\geq$30mg/m^2 equivalent to 0.06X

the MRHD of 493 mg/m^2)) and also caused cycling to stop (≥30mg/m^2 equivalent to 0.06X the MRHD of 493 mg/m^2)

"Pregnancy"
The paragraph that reads

> MULTAC can cause fetal harm when administered to pregnant women. Dronedarone caused marked effects on embryo-fetal development in rats at 100 mg/kg/day such as increased post-implantation losses, reduced fetal and placental weights, various external, visceral and skeletal malformations in most fetuses. At lower dosages, up to 50 mg/kg/day (corresponding to 4.5 times the recommended human therapeutic dose), dronedarone had no effects on the litters (with the exception of a transient minor effect on the bodyweight gain of the pups from D1 to D4 post-partum). Dronedarone had no effects on the mothers up to 30 mg/kg/day. On the contrary, in rabbits, the high dose level (200 mg/kg/day) did not induce any effects to fetuses.

Should be modified to read:

> Dronedarone was teratogenic in rats given oral doses ≥80 mg/kg (480 mg/m2 or approximately 1X the MRHD of 480 mg/m2) with fetuses showing external, visceral and skeletal malformations (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, ectrodactylia, syndactylia, anterior and/or posterior club feet).

It should also be noted in the label that phototoxicity manifested as erythematous reactions was reported in Guinea Pigs given 100 mg/kg/day (800 mg/m^2 or approximately 2X the MRHD)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: Proposed for atrial arrhythmia, dronedarone is an amiodarone analogue lacking the iodine substituents. In in vitro systems, isolated organ culture and whole animal studies have been used to compare this agent to amiodarone. There are many similarities between the effects of the two agents on the ion channels of the heart and the behavior in various non-clinical models of cardiac function that are intended to show anti-arrhythmic potential.

The drug has relatively low oral bioavailability of in the dog 14 % and rat 22%. The volume of distribution is large, from 12 to 66 l/kg with a moderately high systemic

clearance of 2-4 l/h/kg. In all species studied, including human, dronedarone was highly protein bound (>99%). Dronedarone is also highly metabolized with one known active metabolite (SR35021).

In addition to the usual toxicological characterization, dronedarone has also been assessed for immunotoxicologic potential (no significant issues apparent at this time), effect upon thyroid function (incompletely described) and dyslipidosis (occurs to a lesser extent than seen with amiodarone). Mild respiratory effects (decreased respiratory rate and tidal volume) were seen in the single dose respiratory safety pharmacology study. Phototoxicity (mild to moderate at the equivalent of 2X MRHD) has been demonstrated in albino animals. Dronedarone has been demonstrated to bind to melanin. How this will affect phototoxicity is unknown. The sponsor has provided no characterization of the mechanism of the carcinogenic findings or the endocrine effects.

Some aspects of the non-clinical toxicology are puzzling. There are a number of instances where effects do not seem to follow a dose-response. It also appears that there is a lessening or abrogation of effects with extended dosing. That is, effects apparent after 1 month or 3 months of dosing are not evident after 6 months. Yet, plasma levels are no less at 6 months than they were at 3 months.

The sponsor is to be commended on the thoroughness of some aspects of the non-clinical characterization. That is, because of the similarity to amiodarone, immunotoxicity and phototoxicity studies were conducted and comparator compounds (positive controls) appear frequently. An outside review panel was assembled to provide a second opinion on the carcinogenicity studies. Yet the reporting does not do justice to these efforts. For example:

1) There were inconsistencies between the CTD non-clinical summary and the actual study reports.
2) There were inconsistencies within reports where textual numbers were not precisely the same numbers from the summary tables or single animal data
3) There were instances where findings were mentioned within the text of a report for which I could not find tables of numerical summaries.
4) The quality of the Carcinogenicity report by the outside review panel was disappointing at best.

None of these inconsistencies was large or nor do they change the interpretation of the studies in which they were found. The impression that I get is one of haste and a certain degree of carelessness.

B. Pharmacologic activity: Dronedarone has been shown to have properties of beta adrenergic blockade, and cardiac Na, K and Ca channel modulation. However, there is inadequate characterization of the receptor binding properties of both dronedarone and the two major metabolites SR35021 and SR90154. In particular, the possibility of

interaction with the thyroid receptor, hormonal receptors and steroidal receptors should be investigated.

C. Nonclinical safety issues relevant to clinical use : Dronedarone has been shown to be teratogenic, genotoxic, carcinogenic and to disrupt female cyclicity. The target organs of toxicity appear to be the kidney, liver, and gastrointestinal tract. Renal changes were usually without histological correlates and manifested primarily as changes in serum creatinine and serum electrolytes, excreted creatinine and electrolytes. Mild to moderate phototoxicity has been shown non-clinically in rats at a dose of 200 mg/kg (1200 mg/m2 or 2.5x MRHD based on a surface area comparison). Dronedarone does affect thyroid metabolism but this is incompletely defined. Hepatic effects range from elevations in ALT and AST to necrotic foci, possibly too small to cause perceptible changes in liver status tests. Safety pharmacology showed that dronedarone caused decreases in respiratory rate and tidal volume. Dyslipidosis manifested as foamy macrophages occurs but apparently to a lesser extent than seen with amiodarone.

A radiolabel distribution/excretion study showed that the mean concentration of total radioactivity in the heart, liver and lungs was approximately 13, 5 and 20 times higher respectively at 4 hours post-dose on day 14 compared to 24 hours post dose on day 1. This is indicative of tissue accumulation. Comparing 4-hours post-dose at the earliest steady state vs. 4-hour post-dose several weeks later would more clearly address this potential issue.

Cardiac effects are also noted in rats, dogs and macaques. These include such things as decreased heart rate, increased PR interval and increased QT interval. QTc was inconsistently increased, possibly due to the fact that Bazett's formula was uniformly used no matter what the heart rate of the animals. First degree block was reported in rats, dogs and monkeys. Second degree block was also reported in macaques. Increased T wave amplitude was also noted in some studies. Many of the changes can be viewed as extensions of the expected pharmacology. Both dronedarone and the active metabolite SR35021 inhibit the hERG channel with almost the same potency as cisapride, the positive control for the studies (see page 16 of this review, NDA part I).

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

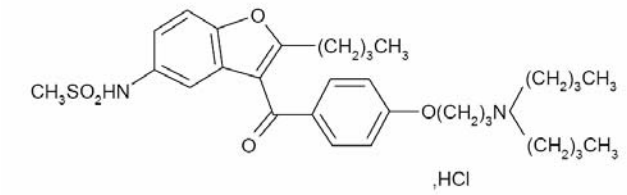
NDA number: 21913
Review number: 1
Sequence number/date/type of submission:
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: Sanofi-Synthelabo
Manufacturer for drug substance:

Reviewer name: Elizabeth Hausner, DVM
Division name: Division of Cardio-Renal Drug Products
HFD #: 110
Review completion date:

Drug:

Trade name: Multac®
Generic name: dronedarone
Code name:
Chemical name:N-{2- butyl- 3-[4-(3- dibutylaminopropoxy) benzoyl] benzofuran- 5-yl}methanesulfonamide, hydrochloride.
CAS registry number:
Molecular formula/molecular weight: $C_{31}H_{44}N_2O_5S,HCl$/ 593.2

Structure:



Relevant INDs/NDAs/DMFs: IND49484

Drug class: antiarrhythmic

Intended clinical population: patients with atrial flutter or atrial fibrillation

Clinical formulation: 400 mg of dronedarone base with inactive ingredients of hypromellose, starch, crospovidone, poloxamer 407, lactose monohydrate, colloidal silicon dioxide, magnesium stearate

Route of administration: oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Studies Reviewed (Many of the pharmacology studies were reviewed in the IND process)

Pharmacology/Safety Pharmacology

In vitro effect of SR35021A on various receptor sites **RS0008950706/01: 685-4-052**
In vitro effects of SR90154 on various receptor sites **RS0008960726/01: 685-4-067**
Effect of SR33589B on the central and autonomic nervous systems in male mice after intravenous administration. **SNX0058**
SNX0054 Effect of SR33589B on the central and autonomic nervous systems in male mice after oral administration
Effects of dronedarone on human cardiac sodium current. Comparison to amiodarone. **CTT103.**
Effects on the hERG channel stably expressed in a mammalian cell line. **PAT0076**
Effects on the hERG channel stably expressed in a mammalian cell line. **PAT0077**
Interaction between SR33589B and Beta-adrenoceptor sites in rat cardiac membranes: in vitro study.**CTT0168** June 1994
Effect of chronic oral SR33589B administration on cardiac beta-adrenoceptor and adenylate cyclase activity in rat. **685-4-043** June 1994.
SR35021A: Effects on the hERG channel stably expressed in a mammalian cell line **PAT0082**
Effects of SR35021A on the action of potential[sic] characteristics of rabbit purkinje fibers. **685-4-054**
Effect of SR33589 in comparison with amiodarone and other class III antiarrhythmic agents on action potential duration in anesthetized rats **685-4-021** November 1991
SR33589B and amiodarone: Electrophysiological actions in anesthetized dogs **CTV0201**
Hemodynamic study after oral administration of SR33589B in the conscious dog. Comparison with amiodarone. **685-4-038** January, 1994
Evaluation of the effects of an acute oral administration of SR33589B on respiratory parameters in unrestrained conscious guinea pigs using whole body plethysmography
Effect of SR33589B on the cardiovascular and respiratory functions in anesthetized dogs after intravenous administration. **CVR0119**
Effect of SR33589B on the cardiovascular and respiratory functions in anesthetized dogs after intraduodenal administration. **CVR0125**
Effect of SR33589B on the hydroelectric balance in rats after intravenous administration **RS0040931005/ION0293**
Effect of SR33589B on the hydroelectric balance in rats after oral administration **ION0217**
Effects of dronedarone on renal function in conscious normotensive rats following repeated oral administration **PGD0133** January 2005
Effect of SR33589B on intestinal transit in mice after oral administration **GIT0029**
Effect of SR33589B on gastric emptying in rats after oral administration. **GIT0030**
Effect of SR3389B on gastric acid secretion in rats after oral administration **GIT0031**
Effects of chronic oral SR33589B administration on plasma thyroid hormone levels in rat. Comparison with amiodarone. **685-4-013**
Phospholipid content of lung and liver in rats treated orally for 14 days with SR3389B and amiodarone **685-4-040**

Pharmacokinetics/Toxicokinetics

Determination of SR33589 and its N-debutyl metabolite (SR35021) in mouse plasma by HPLC-UV following liquid/liquid extraction. **DOS0320**
HPLC-UV assay of SR33589 and its N-debutyl metabolite SR35021 in rat plasma samples **DOS0150**

Validation of an electrospray LC-MS/MS detection method for the quantitative analysis of SR33589 and its metabolite SR35021 in mouse plasma following a Disk SPE (96 well plate) extraction. **DOS0465**
Validation of an LC/MS-MS detection method for the quantitative analysis of SR33589 and its metabolite SR35021 in rat plasma following a Disk SPE (96 well plate) extraction **DOS0444.**
Long term stability of SR33589 and SR35021 in frozen rat plasma. **SPP0010**
Long term stability of SR33589 and SR35021 in frozen dog plasma **SPP0011**
Radioachemical stability and binding to glass and plastic of 14C labeled 33589B in vehicles used for animal studies. **SFA0002**

Pharmacokinetics and absolute bioavailability of SR33589B following single 10 mg/kg intravenous and 30 mg/kg oral administration of (carbonyl-14C)-SR33589B to male and female Sprague-Dawley rats. **Abs0102**
Pharmacokinetic profiles of SR33589B and SR35021 following a single (30 mg/kg) oral or (5 mg/kg) intravenous infusion administration of SR33589B or SR35021A to Beagles. **Abs0209**
The pharmacokinetics of SR33589 following a single 1 mg/kg intravenous administration of (carbonyl-14C)-SR 33589B to male Macaca [sic] monkeys. **ABS0103**
Quantitative tissue distribution and excretion balance of (carbonyl-14C)SR33589B following single oral administration (30 mg/kg) to the male Sprague-Dawley rat. **DIS0045**
Tissue distribution after a single (30 mg/kg) oral administration of (^{14}C-carbonyl) SR335589B to the female Sprague-Dawley rat **DIS0198**
Tissue distribution of radioactivity following a single (30 mg/kg) oral administration of (Carbonyl-^{14}C) SR33589B to the pregnant Sprague-Dawley rat **DIS0231**
Investigation of possible affinity of total radioactivity in melanin containing tissue following a single oral (30 mg/kg) administration of [^{14}C-carbonyl]-SR33589B to pigmented rats. **DIS0407**
Tissue distribution after a single (3 mg/kg) intravenous administration of (^{14}C-carbonyl) SR33589B to the male Sprague-Dawley rat. **DIS0140**

In Vitro Binding of [^{14}-carbonyl]- SR33589B to the Plasma Proteins of Rat, Mouse and Rabbit. **LPR0873**
In vitro binding of SR33589B to rat, dog, monkey maccaca and human plasma proteins. **LPR0508**
In Vitro Binding of [^{14}C- Carbonyl]- SR35021A to the Plasma Proteins of Rat, Dog, Mouse, Rabbit and Human **LPR0876**
Blood distribution and pharmacokinetics of radioactivity following single oral (30 mg/kg) or intravenous (10 mg/kg) administrations of 14C-labelled SR33589B to male and female Sprague Dawley rats Study **LPR0046**
Blood distribution and pharmacokinetics of radioactivity following single oral (25 mg/kg) or single intravenous (2.5 mg/kg) administration of 14C-labeled SR33589B to male Beagle dogs. **LPR0048**
Blood distribution and pharmacokinetics of radioactivity following single intravenous (1mg/kg) administration of 14C-labelled SR33589B to male Macaca monkeys. L**PR0047**
Fetal tissue distribution of radioactivity following a single (100mg/kg) oral administration of (Carbonyl-14C) SR33589B to the pregnant Sprague-Dawley rat. **DPK0054**
Placental transfer of radioactivity after a single oral (60 mg/kg expressed as unsalified compound) administration of [^{14}C-Carbonyl]-SR33589B to the pregnant New Zealand rabbit (Quantitative Whole Body Autoradioagraphy) **PLT0037**
Toxicokinetic study, complementary to the study for effects on embryo-fetal development in the rabbit. **DIV0914**
Interspecies variability in SR33589B metabolism by hepatic microsomal fractions **MIV0143**
Profile and identification of plasma, urine and fecal metabolites following a single and repeated oral administration (75 mg/kg/day) of [14C]-SR33589B to male Swiss CD1 mice. **MET 0462**
Plasma, urinary, tissue and fecal metabolites following single and repeated oral administrations (10 mg/kg/day) of [^{14}C]SR33589B to male Sprague-Dawley rats. **RME0003**
Profile and identification of plasma metabolites following single oral (30 mg/kg) or single intravenous (10 mg/kg) administration of ^{14}C-labeled SR33589B to male and female Sprague-Dawley rats **Met0138**
Profile and identification of urinary metabolites following a single oral (30 mg/kg) or intravenous (10 mg/kg) administration of ^{14}C-labelled SR33589B to male and female Sprague-Dawley rats. **Met0139**
Profile and identification of biliary metabolites following single oral (30 mg/kg) or intravenous (10 mg/kg) administration of 14C labeled SR33589B to male and female Sprague-Dawley rats. **MET0140**

Profile and identification of plasma, urine and fecal metabolites following a single oral administration (60 mg/kg) of [14C]-SR33589B to the female New Zealand white rabbit. **MET0533**
Profile and identification of plasma, urinary and fecal metabolites following single and repeated oral administrations (15 mg/kg/day) of [14C-carbonyl]-SR33589B to male dogs. **RME0009**
Profile and identification of plasma metabolites following a single oral (25 mg/kg) or a single intravenous (2.5 mg/kg) administration of (14C)-SR 33589B to male dogs. **MET0237**
Profile and identification of urinary metabolites following single oral (25 mg/kg) and intravenous (2.5 mg/kg) administrations of (14C)-SR33589B to male dogs. **MET0238**

Profile and identification of plasma metabolites following single intravenous (1 or 8 mg/kg) administration of 14C-labelled SR33589B to male macaca monkeys **MET0141**
Profile and identification of urinary metabolites following single intravenous (2.5 or 8 mg/kg) administration of 14C-labelled SR33589B to male macaca monkeys. **MET0142**

Effects of SR33589B repeated oral administration (375,450 mg/kg/day) during 14 days on various liver monooxygenase activities in CD-1(ICR)BR mouse. **TIN0089**
Effects of a 2-week repeated oral administration of SR33589B (30, 70 and 160 mg/kg/d) on various liver enzyme activities in Sprague-Dawley rats. **TIN0045**
Effect of a 2-week repeated oral administration of SR33589B (25, 60 and 140 mg/kg/d) on various liver enzyme activities in Beagle dogs. **TIN0046**
Pharmacokinetics and excretion balance after single and repeated oral (10 mg/kg/day) administration of [14C-carbonyl]-SR33589B to male Sprague-Dawley rats. **RDS0008**
Excretion balance following a single oral (30 mg/kg) or a single intravenous (10 mg/kg) administration of 14C-labelled SR33589B to male and female Sprague-Dawley rats. **EBA0056**
Enterohepatic recirculation of radioactivity following a single intravenous (10 mg/kg) administration of [^{14}C]-SR33589B to male Sprague-Dawley rats. **MET0464**
Pharmacokinetics and excretion balance after single and repeated oral (15 mg/kg/day) administration of[14C-Carbonyl]SR33589B to male dogs. **RDS0009**
Excretion balance following single oral (25 mg/kg) or single intravenous (2.5 mg/kg) administration of 14C-labelled SR33589B to male Beagle dogs.**EBA0092**
Excretion balance following a single intravenous (2.5 mg/kg) administration of 14C-labelled SR33589B to male macaca monkeys. **EBA0057**
The secretion of total radioactivity in milk of lactating albino rats following a single oral (30 mg/kg) administration of [^{14}C-carbonyl]-SR33589B. **MIL0003**

Toxicology
TXA0224 Single dose oral toxicity study in mice and rats of both sexes(3/31/93 initiated).
TXA0232 Single dose oral toxicity study in mice and rats of both sexes
TXA0231 Single dose intravenous toxicity study in mice and rats of both sexes
TXA0290 Single-dose intravenous toxicity study in mice and rats of both sexes
TXA879 Two-week oral toxicity study in the rat
TXC088 Three -month oral toxicity study in the rat
TXC0986 Six-month oral toxicity study in the rat
DDO0499 4-day intravenous dose-range finding study in the rat
DDO548 Preliminary toxicity study by intravenous infusion to CD rats for seven days
TSA0963 Toxicity study by intravenous infusion to CD rats for four weeks
4-Week intravenous infusion toxicity study in the rat: toxicokinetic data. Complement to the report RS0006950316/01. **TSA0963**
TSA0883 Two-week oral toxicity study in the dog
TXC0886 3-month oral toxicity study in the dog
TXC0970 One-year oral toxicity study in the dog
TSA0885 Two-week intravenous toxicity study in the dog
DDO0549 Preliminary toxicity study by intravenous infusion to beagle dogs
TSA0962 Toxicity study by intravenous infusion to Beagle dogs for four weeks
TSA0962 4-week intravenous infusion toxicity study in the dog: toxicokinetic data

DDO0503 Four-day intravenous dose-ranging study in the macaque
TSA0884 2-week intravenous toxicity study in the macaque

Genetic Toxicology
CEL0593 Ames test-reverse mutation assay on Salmonella typhimurium
Cel0709 Ames Test-Reverse mutation assay on Salmonella typhimurium
FSRFU-LYM0125-EN-E01 In vitro gene mutation assay at the locus TK +/- in mouse lymphoma L5178Y cells
685-3-008 In vitro gene mutation assay at the locus HPRT in Chinese Hamster V79 fibroblasts.
DNA001 In vitro DNA repair assay on rat hepatocytes in primary culture
CEL0536 In vitro DNA repair assay on rat hepatocytes in primary culture
MAF0018 Lymphocyte cytogenetic study
Mut0046 Micronucleus test in vivo genotoxicity study by the oral route in the mouse.

Reproductive and Developmental Toxicology

FER250 Preliminary Segment I study in the rat

Previously Reviewed:

Study of effects on fertility and early embryonic development in CD rat by oral gavage administration **FER0297**
TER0242 Preliminary teratology study in the rat
TER0244 Teratology study in the rat
DD0518 13-day oral dose range-finding study in female rabbits
TER0241 Preliminary teratology study in the rabbit
TER0243 Study of effects on embryo-fetal development in the rabbit.
DPN0295 Study of the effects of SR33589B on pre- and post-natal development (including maternal function) in the rat by oral gavage

Special Toxicology Studies
A four week oral immunotoxicity study in the rat.
Evaluation of phototoxicity and/or photoallergy in the guinea pig. **PHO121-137-141**

Study of the hemolytic potential in vitro. **HEM0009**
Hemolytic potential in vitro **HEM013**
Study of the hemolytic potential in vitro. **HEM0015**
Study of hemolytic potential in vitro. **HEM0026**

Carcinogenicity Studies
CAR0032 An assessment of oncogenic potential in mice following oral administration
CAR0036 A study to assess oncogneic potential in rats following oral administration
EXP0007 Mouse (CAR0032) and rat (CAR0036) carcinogenicity studies. Dronedarone (SR33589B). Scientific advisory panel report.

Studies not reviewed within this submission:

Validation of an electrospray LC-MS/MS detection method for the quantitative analysis of SR33589B and its metabolite SR35021 in guinea pig plasma following a Disk SPE (96 well plate) extraction**DOS0753**
Validation of an electrospray LC-MS/MS detection method for the quantitative analysis of SR33589B and its metabolite SR35021 in rabbit plasma following a Disk SPE (96 well plate) extraction**DOS0613**
Validation of an LC-Ms/MS method for the quantitative analysis of SR33589 and its metabolite SR35021 in dog plasma following a solid phase extraction. **DOS0534**
Determination of SR33589 and its N-monodebutyl metabolite SR35021 in dog plasma by HPLC-UV following liquid-liquid extraction **DOS0255**
HPLC-UV assay of SR33589 and its N-debutyl metabolite, SR35021 in macaca plasma samples. **DOS0149**
Long term stability of SR33589 and its metabolite (SR35021) in frozen spiked mouse plasma samples **SPP0172**

Investigation of long-term stability of SR33589 and its metabolite SR35021 in rat plasma **SPP0195**
Preliminary pharmacokinetic profile of SR33589 and SR35021 after single 2.5 mg/kg intravenous and 100 mg/kg oral administrations of SR33589B to beagle dogs. **APS0032**
Quantitative tissue distribution of total radioactivity in the male albino rat following daily repeated oral (10 mg/kg/day) administrations of [14C-carbonyl]SR33589B(quantitative whole body autoradiography) **RDI0003**
Blood distribution of SR33589B in rat, dog and macaca – in vitro study **LPR0108**

Note: For NDA reviews, all section headings should be included.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary The pharmacology of dronedarone has been explored using cell membrane preparations, various other in vitro methodologies, isolated organ systems and a number of whole animal systems including rats, guinea pigs, rabbits, dogs and pigs. The drug has been demonstrated to possess a number of pharmacologic actions primarily focused on the cardiovascular receptors and cardiac ion channels. The sponsor has also made use of concurrent comparator compounds in many of the pharmacology studies.

Dronedarone has been shown to bind to and inhibit the rapid sodium channel. The decrease in dV/dt_{max} caused by the drug was frequency dependent with rapid onset kinetics. This is characteristic of Class I anti-arrhythmic agents.

In vitro and ex vivo studies showed that dronedarone decreased stimulation of adenylate cyclase activity by both isoprenaline and glucagon but did not effect stimulation resulting from direct acting agents such as sodium fluoride or Forskolin. The drug was thus characterized as a non-competitive β-adrenergic antagonist (Class II anti-arrhythmic).

While dronedarone interacted with a number of the different K+ channels, the effects were not uniform. Concentration –dependent effects were reported for I_{kr}, I_{ks}, $I_{k(Ach)}$ and $I_{kv1.5}$. The sponsor cites these effects as suggestive of a role for dronedarone in cases where increased vagal tone is important, e.g. atrial fibrillation. $I_{kv1.5}$ is considered important in repolarization of human and canine atria. Affinity for this channel is suggested as underlying the increase in atrial effective refractory period seen in anesthetized dogs. It may be noted that dronedarone gave 94.4±1.8% inhibition of the hERG channel expressed in CHO cells at a concentration of 10 μM. Cisparide in the same experiment produced 100% inhibition of the hERG channel at the same concentration. QT interval was consistently prolonged in those studies where ECG data was gathered. The QTc values were generated by Bazette's formula, inappropriate given the heart rate of the animals used. Thus the QTc values were inconsistently elevated. Interaction with K+ channels is characteristic of Class III anti-arrhythmics.

Dronedarone also showed binding to and use-dependent inhibition of L-type calcium channels, a characteristic of Class IV anti-arrhythmic drugs. In vitro this corresponded to decreased shortening of isolated ventricular cells. In some of the whole animal studies were ECG data was gathered, changes were manifested in the PR interval and the T-wave amplitude.

Dronedarone decreased sinus rhythm by a variety of mechanisms including decreased rate of diastolic depolarization and lengthening of the APD ($I_{Ca\text{-}L}$ and I_k). Decreased heart rate was seen when dronedarone was given to rats and dogs. Other cardiac data provided showed increases in the Wenckeback cycle length, AH and PQ interval, suggestive of changes in AV nodal conductance.

There were several safety pharmacology studies that reported decreases in peripheral resistance. The calcium channel effects may have contributed to this. In vitro studies indicated mild interaction of the drug with α1, β1 and β2 adrenergic receptors. How much this contributed to the peripheral resistance decreases in vivo is uncertain.

The circulating metabolite SR35021A was examined in isolation for its hemodynamic effects when given intravenously to anesthetized mixed-breed dogs (CVT0209). Observation after 1 mg/kg lasted 1 hour, observation after 3 mg/kg lasted 2 hours as did the observation after 10 mg/kg.

SR35021A given intravenously (sponsor's numbers)

	Baseline values mean±sem	1 mg/kg	3 mg/kg	10 mg/kg	Vehicle range
HR bpm	122±6	-8%	6%	38% (>120)**	+6 to +12%
TPR $10^{2.}$.dynes.s/cm^5	31±1	7%	12%	-46%(15)**	-3 to +19%
SBP mm Hg	176±6	-5%	3%	-12%(60)*	-6 to +7%
DBP mm Hg	124±4	3%	6%	-15%(10)*	+3 to +6%
MBP mm Hg	146±4	3%	4%	-13%(15)*	-3 to +7%
PADP mm Hg	10±1	10%	19%(>40)	25%(20)	22 to 30%
LVEDP mm Hg	5±1	40%(5)	+29%(70) -23%(10)	90%(110)	-20 to +11%
CO (l/min)	3.8±0.2	-5%	-9%	61%(10)**	-11 to +3%
Carotid BF (ml/min)	195±23	-24% (>60)*	-27%(120)*	45%(10)	-17 to +23%
LVW	8.1±0.7	-9%	-9%	39% (10)*	6 to 9%
SV	32±2	3%	-9%	14%(5) -24%(120)*	-16 to -8%
RVEF %	42±3	-3%	-21%	-25% (110)**	NC

( ) total duration of effects *p<0.05, **p<0.01

The low dose had minimal effect on the hemodynamic parameters. The MD of 3 mg/kg produced a 21% decrease in RVEF and a 27% decrease in carotid blood flow for approximately 2 hours. The HD of 10 mg/kg produced a negative inotropic effect (-12%) and a 25% decrease in RVEF for ~110 minutes accompanied by a corresponding decrease in stroke volume. The increased HR (38%) and 46% decrease in total peripheral resistance caused a brief (10 minute) increase in cardiac output.

Detrimental ECG changes were also reported with administration of SR35021: extrasystoles (PVCs?) were seen in 1/5 (LD), 5/5 (MD) and 3/5 (HD). Most of the animals showed multiple PVCs. The vehicle used (PEG 400) did not cause the reported changes.

Most of the ECG changes are expected as extensions of the primary pharmacology.
From the data available I am not sure if both the parent drug and SR35021A or if only SR35021A are responsible for the undesirable ECG changes (AV block, extrasystoles, Torsade de Pointe). As coronary blood flow was not measured in the hemodynamic assessment of the metabolite, the same can also be said for the effect measured in previous intravenous administration studies.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: The mechanism of action appears to be due to the combination of the various ion channel effects. It cannot be determined from the available information if one mechanism predominates. Another consideration is that some single mechanism as yet undescribed is responsible for the pharmacologic action.

In vitro receptor binding for the parent drug and SR35021 and SR90154, the two main metabolites, is poorly characterized. No data was presented beyond cardiac ion channels and cardiovascular receptors.

IC50 (μmol/l) of dronedarone on different ion channels in guinea pig heart

	I_{kr}	I_{Ks}	I_{Kl}	I_f	I_{KACh}	$I_{Ca\text{-}L}$	$I_{Ca\text{-}T}$
dronedarone	≤3	~10	≥30	≥30	~0.01	0.18	>30

IC50(μmol/l) of dronedarone on human hERG channel

	hERG(expressed in CHO cells)	hERG (expressed in HEK cells)	$K_{v1.5}$
dronedarone	0.53	0.059	2.7

IC50 values for other targets

Target	dronedarone	Amiodarone
Calmodulin-dependent cyclic nucleotide phosphodiesterase	0.89 μmol/l	0.12μmol/l
Ca_L	546±13nmol/l	173±5 nmol/l
Na+ channel (batrachotoxin) Heart brain	 2060±104nmol/l 413±46nmol/l	 1654±227nmol/l 645±105 nmol/l

Drug activity related to proposed indication: Various in vitro, ex vivo and in vivo studies have shown that dronedarone can alter the characteristics of the ECG, action potentials, Wenckebach cycle and cardiac function parameters. The results are supposed to be indicative of anti-arrhythmic potential in the human. The various ion channels and receptors that the sponsor states to be affected by dronedarone are the mechanisms usually associated with anti-arrhythmic effects.

In the atrium, dronedarone decreased the spontaneous sinus rhythm, action potential amplitude and lengthened the action potential duration in the guinea pig sinoatrial node. In the ventricles, dronedarone decreased dV/dt max of the AP. Cardiac contraction was reduced. Dronedarone was also demonstrated to prolong the Wenckeback cycle length (reviewed in the IND) and slightly prolong the effective refractory period.

In the sponsor's proposed labeling, they also mention that dronedarone has vasodilatory properties and increased coronary blood flow (CBF). A study using isolated rat hearts in Langendorf preparations showed an increase in coronary blood flow of 20-30% compared to baseline (report 685-4-019). Anesthetized dogs given intravenous infusions of dronedarone of 1 and 5 mg/kg, 30 minutes apart showed a decrease in coronary resistance. At rest, 1 mg/kg caused a non-significant increase in (CBF). The 5 mg/kg dose caused a transient fall in MVO_2 (-69% at 35 minutes and -7% at 60 minutes post infusion) and a transient rise in CBF of +54% (32 minutes post-infusion and +46% at 45 minutes post-infusion (report 685-4-006). Decreased MVO2 may be associated with the decreased HR seen.

2.6.2.3 Secondary pharmacodynamics

In vitro effect of SR35021A on various receptor sites RS0008950706/01: 685-4-052 Using material from guinea pigs and Sprague-Dawley rats, the metabolite was used in competition experiments to examine binding of radioligands in a limited panel of receptors. Weak activity was reported for β1 and β2 adrenergic (≤10% inhibition at 3 μM), α1 and α2 adrenergic, purinergic A1 and cholinergic muscarinic receptor sites.

In vitro effects of SR90154 on various receptor sites RS0008960726/01: 685-4-067
SR90154 was examined in competition studies on specific binding to a limited array of receptors in brain, heart and lung membranes. This metabolite showed mild receptor activity for the α1-adrenergic receptor (14±1% inhibition at 30 μM), cholinergic muscarinic (19±3% at 30 μM), purinergic A1 (16±1% inhibition at 30 μM), L type Ca++ channel (10±2% inhibition at 30 μM), Na+ channel (17±3% inhibition at 30μM), ATP-dependent K+ channel (31±1% inhibition at 30 μM)

2.6.2.4 Safety pharmacology

Summary: the effects of dronedarone were assessed in models of neurologic, cardiovascular, pulmonary , renal and gastrointestinal function.

Neurological effects: A single intravenous injection of 10 mg/kg caused a non-significant decrease in the activity level of mice. A single oral dose of ≥200 mg/kg caused a decrease in spontaneous motor activity in rats. A single oral dose of 800 mg/kg caused sedation.

Cardiovascular effects: The sponsor performed extensive pharmacological investigations into the effect of the drug on the cardiovascular system. A very few of these tests are summarized in the table below.

Summary of some Cardiovascular Safety Comparisons

Parameter studied	Comparator compound results	Dronedarone or metabolite result
Inhibition of atrial sodium channels	Amiodarone: 80±7% @30 μM	97±4% @ 3 μM
Inhibition of inward hERG (CHO cells)	Cisapride 100% @ 10 μM	94.4±1.8% @10 μM
β-adrenoceptor inhibition IC50 nM	Amiodarone 8650±2040 (±)propranolol 11.6±1.1	1755±360
Inhibition of inward hERG (CHO)	Cisapride 100% @ 10μM	SR35021A 91.8±3.7% @10μM
APD90 in anesthetized rats	Amiodarone 83% @ 30 mg/kg Cisapride 41% @ 30 mg/kg	39% @ 10 mg/kg. 60% of the rats experienced an AV block

Cardiovascular effects were also examined in anesthetized and conscious dogs. In anesthetized dogs, IV amiodarone was used as a comparator for intravenous dronedarone. Dronedarone caused approximately twice the decrease in heart rate compared to amiodarone based on percentage of means. Dronedarone also caused substantially greater increase in the PQ interval (42 vs 11, units not given) based on percentage of means. The conscious dogs received oral amiodarone or dronedarone. Plasma levels of drug were measured. There was no difference in plasma levels between 12.5 and 25 mg/kg (presented graphically, 0.3-0.4 nmol/ml) but there was an increase from 25 to 50 mg/kg/day (peaking at 3 nmol/ml). The sponsor showed a dose-related increase in heart rate (opposite to the decreases seen in toxicology studies): an increase of 27% at the LD of 12.5 mg/kg lasting for 45 minutes up to a 39% increase at the HD of 50 mg/kg lasting for 420 minutes. Dronedarone produced greater than usual changes in the heart rate and contractility in this study, also based on percentage of means. The results are summarized in the sponsor's table below. It could be questioned whether a larger sample size would have decreased the differences between the two drugs.

Sponsor's summary of dronedarone results

	12.5 mg/kg (n=5)		25 mg/kg (n=8)		50 mg/kg(n=6)	
	Control values	Maximal Var.%	Control values	Maximal Var.%	Control values	Maximal Var.%
HR(bpm)	75±7	+27(45)	75±4	+24* (330)	77±4	+39**(420)
DAP(mm HG)	79±5	-6	78±3	+6	81±6	-16(>420)
SAP(mmHG)	131±8	-8	126±3	-4	125±8	-17(>420)
MAP(mmHG)	96±6	-7	94±2	-4	96±6	+16
LVSP(mmHG)	138±7	-7	133±3	-7	137±5	-17*(>420)
LVEDP(mmHG)	12±3	-22	11±1	+17	14±2	-7
Cont(s^{-1})	32±3	+8	28±1	+9	26±1	-32**(>420)
Relax (s^{-1})	23±1	+16	22±1	+7	21±2	+7(375)

Control values were the mean±sem of the seven measurements before administration for all dogs of each group. Treatment-induced variations were calculated as percentage of means.
( ) total duration of effect in minutes
*p<0.05, **p<0.01 by the Mann Whitney U test

Sponsor's summary of amiodarone results

	25 mg/kg (n=6)		50 mg/kg(n=6)	
	Control values	Maximal Var.%	Control values	Maximal Var.%
HR(bpm)	77±4	+10	75±4	+2
DAP(mm HG)	84±4	-9	80±6	+8
SAP(mmHG)	131±5	-9	131±6	+3
MAP(mmHG)	99±4	-8	97±6	+5
LVSP(mmHG)	145±4	-6	139±9	-8
LVEDP(mmHG)	14±2	-22	14±2	+13
Cont(s^{-1})	29±2	+13	28±2	-13
Relax (s^{-1})	22±2	+14	21±1	+13

Control values were the mean±sem of the seven measurements before administration for all dogs of each group. Treatment-induced variations were calculated as percentage of means.
( ) total duration of effect in minutes
*p<0.05, **p<0.01 by the Mann Whitney U test

Pulmonary effects: were assessed in guinea pigs using whole body plethysmography. Theophylline was the positive control. At the highest dose of 100 mg/kg dronedarone caused a mild decrease in respiratory rate from 30 to 105 minutes after oral dosing (-11 to -16 mean change from baseline vs a change of -5 to -8 in the control group). Despite large variability, a doses of 30 mg/kg caused a depression of tidal volume of -6 to -23 mean change from baseline while theophylline caused a change from 30-60 minutes of -17 to -62 mean change from basleine.

Another study of both respiratory and cardiovascular effects following intravenous administration of dronedarone to anesthetized dogs showed dose-related effects on essentially

every parameter assessed. Mean blood pressure, heart rate, total peripheral resistance and contractility showed decreases with increasing dose. The PR interval increased as did the QT interval. Respiratory effects were also apparent with a dose-related increase in respiratory frequency and flow. Tidal volume showed a lot of variability. The results are summarized in tabular form on page 28 of this review. Oral, IV and intraduodenal effects are compared in the reviewer's table below

Intraduodenal administration of drug produced decreases in mean blood pressure and variable increases in heart rate. LV stroke volume and CO correspondingly increased with a decrease in total peripheral resistance. The respiratory changes were similar to those already recorded in that respiratory frequency and flow increased. Tidal volume again showed variability with a decrease at the HD.

Comparison of respiratory effects with different routes of administration of dronedarone (respiratory effects were not measured in dogs following oral administration)

	IV	Intraduodenal
Doses mg/kg	1,5	12.5, 25
Respiratory frequency (Cycles/min)	↑+9-15	↑+9-14
Respiratory flow(l/min)	↑up to 89%, parallel to respiratory frequency	↑51-127%, parallel to respiratory frequency
Tidal volume	↓~13% from 10-30 minutes	↓10-19%

Comparison of cardiovascular effects with different routes of administration of dronedarone

	Oral	IV	Intraduodenal
Doses mg/kg	12.5, 25, 50	1,5	12.5, 25
Heart rate	+45-420%	0- ↓29 bpm	+0-12 bpm
DAP mm Hg	+6 to -16		
SAP mm Hg	-4 to -17		
MAP mm Hg	-4 to +16	↓ to 32 mm Hg	↓ to 13 mm Hg
LVSP mm Hg	-7 to -17	↓parallel to blood pressure	↑ 27%
Contractility (s^{-1})	+9 to -32		
Cardiac output		↑early up to 35%	↑to 30%

The ECG changes of increased PR interval and increased QT are consistent with the toxicology studies. The variability of some results may be due to the vagaries of oral and intraduodenal absorption, and variations in individual metabolism. There is also a consideration that these tend to be acute, one exposure studies, versus the effects seen in the repeat dose toxicology studies.

Renal effects: After IV administration, females showed a dose-related increase in specific gravity (1.02-1.025, $p<0.01$) with no effect in males. Both sexes showed an increase in excreted K+ concentration (males 3-5 mM over control values; females 1-30 mM over control values). While creatinine clearance and concentration was unaffected in males, females showed a dose-related

increase in both parameters: Cr_{Cl} 28 to 33% and Cr_{conc} 1.7 – 2.7. Both sexes were also reported to have blood in the urine. Following oral administration there were some slight changes of questionable biological significance such a decrease in excreted creatinine in the HD of both sexes. The quantity of excreted potassium decreased in both sexes at the HD. Decreased creatinine clearance was seen in both sexes. Correspondingly the concentration of serum creatinine increased in both sexes. Serum sodium and potassium increased only in the females. Hematuria was not reported in this study as it was in the IV administration study. Alterations in creatinine and excreted electrolytes were also noted in the toxicology studies.

Gastrointestinal effects: Given orally to mice, dronedarone at the LD (100 mg/kg) and HD (1000 mg/kg) caused increases in intestinal transit time of 14% and 36% ($p \leq 0.05$) respectively. Given orally to rats, 100 mg/kg caused a decrease in gastric emptying of 10% while the HD of 300 mg/kg caused a decrease of 53%. Thus in these two species the same drug caused opposite effects upon the gastrointestinal system. Acid secretion was measured in rats after a single oral dose of 100 mg/kg dronedarone. There was a 13%(NS) increase in the volume of acid secretion.

INDIVIDUAL STUDIES

Neurological effects:
Effect of SR33589B on the central and autonomic nervous systems in male mice after intravenous administration. ***SNX0058*** June-July 1993. Male OF1 mice 10-12/group were given 0,3 or 10 mg/kg at an injection rate of 1 ml/min. Effect of the drug on central and autonomic NS was assessed by modified Irwin's test, body weight changes (7 day follow up) , body temperature, muscle tone and motor coordination and spontaneous motor activity. The behavior, body temp, and musculoskeletal parameters were observed 15, 30, 60 and 120 minutes after dosing. Spontaneous motor activity was performed 5 minutes after administration over a 30 minute period. There were no apparent differences in body weight and no unscheduled mortality was reported. No differences in behavior were recorded between the different groups. A slight decrease in body temperature was noted in all groups for the 2 hours of observation. No differences between groups were noted in the tests of motor coordination. There was a non-significantly lower level of activity in the 10 mg/kg group at the 3 timepoints of measurement.

SNX0054 *Effect of SR33589B on the central and autonomic nervous systems in male mice after oral administration. October-November, 1992*
Test A: doses of 0, 50, 100, 200, 400 and 800 mg/kg
Tests B,C,D: doses of 0, 100, 200 mg/kg The vehicle used for all tests was 5% gum Arabic

Male OF1 mice were used, 10-12/group. The tests were A. Behavior(modified Irwin's) and body weight (7 days) B. body temperature C. Muscle tone and motor coordination D. spontaneous motor activity. For A,B and C observations were performed at 30 , 60, 180 and 360 minutes. There was a 7 day follow-up for test A. Test D was performed at 30, 60 and 180 minutes after administration over a 30 minutes period for each evaluation time.

No unscheduled mortality was reported.

There was no significant difference in weight gain between the control group and doses up to an including 400 mg/kg. The 800 mg/kg group gained on average 4% less than the control group. The difference in rate of gain was apparent from day 3.
Doses ≥200 mg/kg caused a decrease in spontaneous motor activity. A dose of 800 mg/kg caused sedation.

Cardiovascular effects:
Effects of dronedarone on human cardiac sodium current. Comparison to amiodarone. ***CTT103.***

Human atrial myocytes obtained during surgery were dissociated and sodium currents obtained from whole cells, at room temperature by patch clamp technique. Sodium currents were obtained in the presence of TEA and 4-AP with low extracellular concentration of sodium ions (27 mM NaCl). Test substances were added to the extracellular solution and perfused on the cells for 7-10 minutes to obtain the maximum effect.

The sponsor's table is reproduced below:
Inhibition of percentages of I_{Na} by dronedarone and amiodarone

	0.3 μM	3 μM	30 μM
Dronedarone	23±10 [a]	97±4 [b,c]	
amiodarone		41±11[a,c]	80±7[b]

a: not significant (0.3μM dronedarone vs 3 μM amiodarone)
b: not significant (3μM dronedarone vs 30 μM amiodarone)
c: p<0.003 (3 μM dronedarone vs 3 μM amiodarone)

Dronedarone produced a greater inhibition of the atrial sodium channels at a lower concentration than amiodarone.

Effects on the hERG channel stably expressed in a mammalian cell line. PAT0076

Chinese hamster ovary (CHO) cells stably transfected to express the hERG channel were examined with the patch clamp technique. Dronedarone in 0.1% DMSO at concentrations of 0.01, 0.1,1 and 10μmol/l was added to the test system. Cisapride at 10 μmol/l was the positive control.

Summary of inhibition of inward tail of hERG as a percent of the vehicle control

	IC50	Drug concentration (μmol/l)		
		0.1	1	10
Dronedarone	531±27 nmol/l; (315±16 ng/ml)	15.9±5.8%	65.6±7.6	94.4±1.8%
cisapride				100%

Under the conditions of the assay, dronedarone caused an inhibition of the hERG channel at a level similar to that of cisapride.

Effects on the hERG channel stably expressed in a mammalian cell line. ***PAT0077***

Chinese hamster ovary (CHO) cells stably transfected to express the hERG channel were examined with the patch clamp technique. Amiodarone was used at concentrations of 0.01, 0.1, 1 and 5 μmol/l. Cisapride was again the positive control.

Summary of inhibition of inward tail of hERG as a percent of the vehicle control

	IC50	Drug concentration μmol/l			
		0.1	1	5	10
amiodarone	1.36±0.18μmol/ 928±123 ng/ml	15.0±4.8%	41.4±4.3%	75±4.5	
cisapride					100%

Under the conditions of the study amiodarone was less potent than dronedarone in inhibiting the Ikr channel based upon IC_{50} comparison.

*Interaction between SR33589B and Beta-adrenoceptor sites in rat cardiac membranes: in vitro study.****CTT0168*** *June 1994*

This study examined the effects of SR33589B in DMSO (10^{-8}M to 10^{-4}M) in competition, saturation and dissociation experiments on the specific binding of [^{125}I]-(-)iodocyanopindolol ([^{125}I]-(-)-CYP to rat cardiac membranes. Amiodarone and ±propranolol were used as comparators. The conditions used were Tris buffer (50 mM) pH7.4 containing 10mM $MgCl_2$ and cardiac membranes (100 μg protein) incubated for 1 hour at 37ºC. Total bound radioactivity was measured after filtration and rinsing. Specific binding was defined in the presence of 2μM(-)-alprenolol (dissociation was induced by this addition).

Dronedarone had an IC50 for this binding somewhere between that of amiodarone and ±propranolol (approximately 4 times the affinity of amiodarone). Both amiodarone and dronedarone showed non-competitive interaction with the beta-adrenoceptor sites.

The sponsor's table is reproduced below.

Sponsor's Summary of responses: Dose-response curves: IC_{50} nM

	SR33589B	Amiodarone	(±)propranolol
[^{125}I]-(-)-CYP	1755±360	8650±2040	11.6±1.1

n_H included between 0.86 and 0.93

Effect of chronic oral SR33589B administration on cardiac beta-adrenoceptor and adenylate cyclase activity in rat. ***685-4-043*** *June 1994.*

Male rats (10/group) were treated orally with SR33589B or amiodarone at 0(vehicle), 50, 100 or 150 mg/kg/day in gum arabic suspensions for 14 days. Measurements made included 1) β-adrenoceptor sites by [^{125}I]-(-)-iodocyanopindolol binding, 2) adenylate cyclase activity stimulated by GTP, GppNHp,3)±isoprenaline, 4)glucagon, 5) NaF and 6) forskolin in cardiac membranes.

Neither amiodarone nor dronedarone appeared to affect the catalytic or regulatory portion of the enzyme complex. However, both decreased the adenylate cyclase activity stimulated by isoprenaline and glucagons to approximately the same extent.

The sponsor's results are reproduced below.
Mean ±SEM unless otherwise specified.

Group	Adenylate cyclase activity (pmol cAMP/min/mg prot)						
	basal	GTP (0.01mM)	GppNHp (0.1mM)	NaF (10mM)	Forskolin (0.1mM)	Isoprenaline (1mM)	Glucagon (0.01mM)
control	14.8±0.9	25.8±1.7[a]	98±4[a]	103±5[a]	442±14[b]	60.3±1.6[b]	46.8±1.6[b]
SR33589B							
50 mg/kg	15.5± 0.9[a]	23.0±1.4	95±5	96±5	426±31	51.0±1.9[a]**	38.1±2.5*
100 mg/kg	15.6±0.7	24.3±0.6	99±4	113±8	490±20	51.0±2.4**	38.6±2.6*
150 mg/kg	15.0± 1.7[a]	23.5±1.1[a]	93±6	114±7	440±9	41.4±3.7***	33.3±2.8[b]***
Amiodarone							
50 mg/kg	15.8±1.1	24.9±1.3	98±3[a]	112±8	458±21	55.0±6.1	45.0±5.7
100 mg/kg	13.3± 0.8[a]	23.3±2.0[a]	86±6	93±2	402±33	43.8±4.5**	34.5±3.8[a]*
150 mg/kg	15.0±1.3	22.7±1.0	84±2	88±2[a]	406±28	37.8±3.2***	30.7±3.7**

*p<0.05, **p<0.01, ***p<0.001 compared to the control group. [a], one value rejected or not determined; [b], two values rejected

SR35021A: *Effects on the hERG channel stably expressed in a mammalian cell line PAT0082*

The hERG channel was transfected into Chinese hamster ovary (CHO) cells. The test material was dissolved in DMSO, applied at concentrations of 0.01, 0.1, 1 and 10 µmol/l and tested by patch clamp technique. Cisapride was used as positive control.

SR35021A blocked the hERG channel as summarized below and with an IC50 of 1.01±0.06 µmol/l.

Concentration of applied material (µmol/l)	0.1	1	10
SR35021A: Inhibition of hERG	4.8±2.9 %	50.6±3.5%	91.8±3.7%
Cisapride: inhibition of hERG			100%

Effects of SR35021A on the action of potential[sic] *characteristics of rabbit purkinje fibers.* ***685-4-054***

The isolated purkinje fibers were superfused with Tyrode's solution (36ºC) and stimulated at 60 beats/minute. The synthesized metabolite was tested at concentrations of 3 and 10 µM. AP parameters were evaluated before and at the end of the 30 and 60 minutes superfusion for 3µM SR35021A and at the time 25-60 minutes just before the occurrence of conduction disturbance for 10µM test article.

Results for the 2 different concentrations were taken at 2 different times due to conduction velocity disturbances.

Reproduction of sponsor's results

	AP variations (Δ%)							
	n	RP	APA	dV/dt max	APD50	APD70	APD90	CT
3µM(60 min)	5	0	-2	-11	-10*	-1	+3*	+4
10 µM(45±7 min)	4	-4*	-22**	-80*	+5	+3	+9	+37

*p<0.05, **p<0.01 versus control;Δ% =percentage variation of control value; RP=resting potential; APA= AP amplitude; dV/dT max= maximum rate of depolarization; APD=action potential duration at 50, 70 or 90% of depolarization; CT= conduction time

The sponsor did not offer an explanation as to the different times of determination for the different concentrations.

Reproduction of sponsor's results

	ERP/APD ratios				
	ERP (ms)	APD70 (ms)	APD90 (ms)	ERP/APD/70	ERP/APD90
Control n=4 3µM 60 min	236±14 244±15**	199±16 196±22	252±10 267±10	1.20±0.07 1.27±0.12	0.94±0.05 0.91±0.04
Control n=5 10µM 228 min	223±14 219±7	200±14 198±4	228±14 235±5	1.12±0.03 1.11±0.02	0.98±0.02 0.93±0.02

ERP= effective refractory period

In rabbit purkinje fibers, SR35021 caused a decrease in resting potential at 10 μM and an apparently dose related decrease in dV/dt max and AP amplitude. The sponsor concluded that the in vitro electrophysiological profile of SR35021A in rabbit purkinje fibers was similar to that of dronedarone.

Effect of SR33589 in comparison with amiodarone and other class III antiarrhythmic agents on action potential duration in anesthetized rats ***685-4-021*** *November 1991*

The effect of dronedarone and several class III antiarrhythmic agents (amiodarone, d-sotalol,UK68798, RS87337) on the action potential duration was measured in sodium pentobarbital anesthetized rats(SD) maintained with artificial ventilation. The drugs were given intravenously.

Dronedarone, amiodarone and D-sotalol caused dose-related increases in APD_{90} . At the highest dose of dronedarone, 3 out of 5 animals developed an A-V block that resulted in mortality. The sponsor's results are shown below.

Drugs i.v.	Dose (mg/kg)	APD_{90} (max Δ%)	HR (Δ%)	APDc (max Δ%/ $\sqrt{HR}$)
PEG/H_2O (4/1)	Control	+ 1	- 8	0.4
SR 33589 B	0.3	+14	- 6	5.6
A	1	+25	- 5	11.4
B	3	+27	- 9	9.0
A	10	+39 (60% AVB)	-11	11.8
AMIODARONE	1	+11	- 5	4.9
	10	+16	- 9	5.3
	20	+61	-23	12.7
	30	+83	-23	17.3
D-SOTALOL	1	+24	-13	6.7
	10	+31	-18	7.3
	30	+41	-23	8.5
UK 68798	0.01	+ 3	- 6	1.2
	0.1	+ 1	-13	0.3
RS 87337	1	+ 6	- 6	2.4
	10	+29	-27	5.6

SR33589B and amiodarone: Electrophysiological actions in anesthetized dogs ***CTV0201***

Mongrel dogs (6/sex for amiodarone and dronedarone and 5/sex for control group) were given 5+5mg/kg amiodarone (aqueous solution) or 5+5 mg/kg dronedarone(1% PEG with water) intravenously in 2 injections at 60 minute intervals. Anesthesia was maintained with 0.8% α-chloralose.

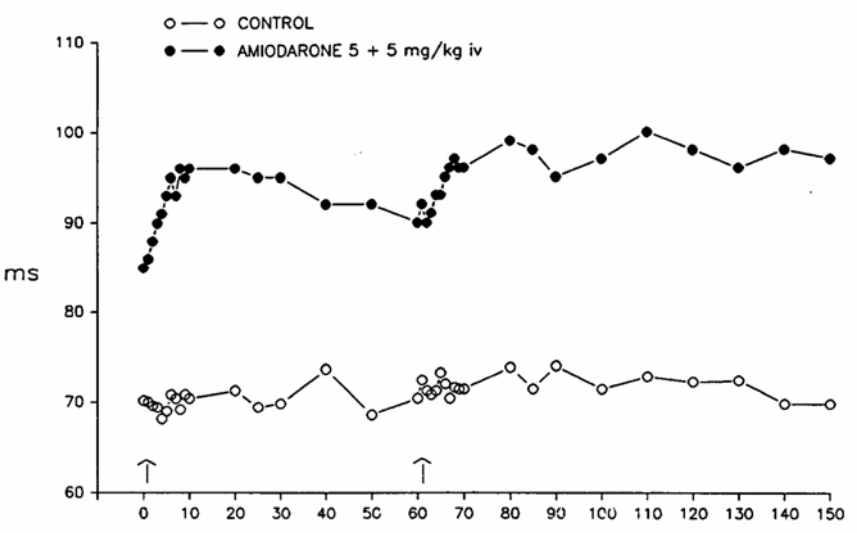
The sponsor's results are shown here.

NaCl 0.9 % (n = 5)					
Doses i.v.	4 ml		+ 4 ml		
Time (min)	10	50	+ 10	+50	+90
SCL	2	5	12	19	14
Heart rate	-4	-7	-12	-18	-12
AH	0	-2	2	4	-1
HV	4	3	2	4	6
WENCK	1	2	-1	2	5
ARP Func.	-2	1	1	0	0
Eff.	0	7	9	2	10
AVNRP Rel.	2	2	0	1	2
Func.	1	3	4	5	9*
Eff.	-2	3	3	-9	0
VRP Func.	2	5	4	8*	8*
Eff.	1	5*	3	6*	10*
L.II PQ	-2	0	2	1	2
QRS	1	3	4	4	4
QTb	3	8**	8	10*	14*
QTc	1	5	2	1	7

Dog	Amiodarone (n = 6)				
Doses i.v.	5 mg/kg		+ 5 mg/kg iv		
Time (min)	10	50	+ 10	+50	+90
SCL	13*	26	33	51*	57*
Heart rate	-12*	-19*	-23	-32*	-34*
AH	13	9	14	18*	15
HV	9	12	14	15	17
WENCK	31**	31*	58***	61**	81**
ARP Func.	13**	9**	17**	14*	12
Eff.	13*	10*	18***	14*	11
AVNRP Rel.	4	5	7*	5	5
Func.	19**	17***	35**	43**	44**
Eff.	26	19*	49*	64**	65*
VRP Func.	6	9**	15**	17**	17**
Eff.	6*	11**	17**	19**	20***
L.II PQ	11	7	12	12	12
QRS	-1	3*	4	4	5
QTb	5	13*	15*	24*	30*
QTc	-2	1	0	1	4

Dog	SR 33589B		(n = 6)		
Doses i.v.	5 mg/kg iv		+ 5 mg/kg iv		
Time (min)	10	50	+ 10	+50	+90
SCL	38***	48**	59***	59***	61**
Heart rate	-27***	-31***	-36***	-37***	-37***
AH	60**	49**	65***	51***	39***
HV	1	3	12	9	10
WENCK	83***	78***	112***	102***	91***
ARP Func.	15**	9**	11*	13	12*
Eff.	19	8	14	11	6
AVNRP Rel.	15	14	16	15	16
Func.	59**	44*	66**	53*	32
Eff.	105***	96**	125***	110***	100**
VRP Func.	7	8	13*	13	15*
Eff.	20**	22**	23**	25*	23**
L.II PQ	42*	35**	51**	42**	33**
QRS	4*	4	6	1	6
QTb	7*	17**	19**	19*	23**
QTc	-9**	-4	-6*	-6	-2

Some results were also presented graphically. There were some parameters where the control and treated groups started at different places. An example is shown below in the effect on AH interval.

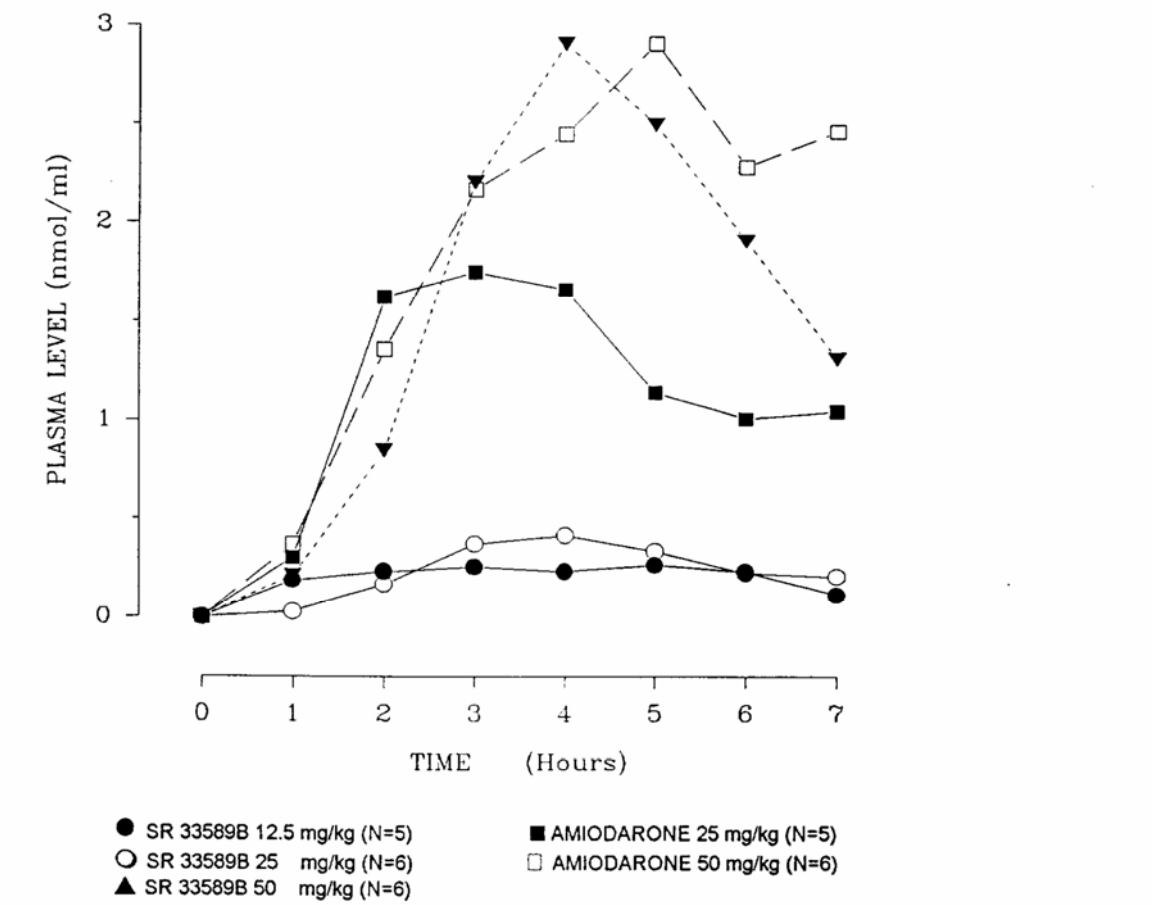


Both agents decreased HR and increased AH intervals. Wenckebach cycle time was also increased as was ARP, PQ and QTb intervals. Dronedarone appeared to affect PQ interval more than did amiodarone.

Hemodynamic study after oral administration of SR33589B in the conscious dog. Comparison with amiodarone. ***685-4-038*** *January, 1994*

Five to 8 mongrels per dose were given either SR33589B (12.5, 25 and 50 mg/kg single doses) or amiodarone (25 and 50 mg/kg single doses) in gelatin capsules. A variety of cardiovascular parameters were measured in these instrumented dogs both before and after drug administration. Effects were observed every 15 minutes after dosing for 7 hours.

Plasma level drug exposure was measured. There was no difference on the plasma levels of dronedarone after 12.5 and 25 mg/kg as shown below.



The sponsor's summary is reproduced below.

Sponsor's summary of dronedarone results

	12.5 mg/kg (n=5)		25 mg/kg (n=8)		50 mg/kg(n=6)	
	Control values	Maximal Var.%	Control values	Maximal Var.%	Control values	Maximal Var.%
HR(bpm)	75±7	+27(45)	75±4	+24* (330)	77±4	+39**(420)
DAP(mm HG)	79±5	-6	78±3	+6	81±6	-16(>420)
SAP(mmHG)	131±8	-8	126±3	-4	125±8	-17(>420)
MAP(mmHG)	96±6	-7	94±2	-4	96±6	+16
LVSP(mmHG)	138±7	-7	133±3	-7	137±5	-17*(>420)
LVEDP(mmHG)	12±3	-22	11±1	+17	14±2	-7
Cont(s^{-1})	32±3	+8	28±1	+9	26±1	-32**(>420)
Relax (s^{-1})	23±1	+16	22±1	+7	21±2	+7(375)

Control values were the mean±sem of the seven measurements before administration for all dogs of each group. Treatment-induced variations were calculated as percentage of means.
( ) total duration of effect in minutes
*p<0.05, **p<0.01 by the Mann Whitney U test

Sponsor's summary of amiodarone results

	25 mg/kg (n=6)		50 mg/kg(n=6)	
	Control values	Maximal Var.%	Control values	Maximal Var.%
HR(bpm)	77±4	+10	75±4	+2
DAP(mm HG)	84±4	-9	80±6	+8
SAP(mmHG)	131±5	-9	131±6	+3
MAP(mmHG)	99±4	-8	97±6	+5
LVSP(mmHG)	145±4	-6	139±9	-8
LVEDP(mmHG)	14±2	-22	14±2	+13
Cont(s^{-1})	29±2	+13	28±2	-13
Relax (s^{-1})	22±2	+14	21±1	+13

Control values were the mean±sem of the seven measurements before administration for all dogs of each group. Treatment-induced variations were calculated as percentage of means.
( ) total duration of effect in minutes
*p<0.05, **p<0.01 by the Mann Whitney U test

The MD of dronedarone caused a significant increase in HR with no significant effect on blood pressure. The HD, 50 mg/kg of dronedarone, caused an increase in HR with a trend of decreased blood pressure.
The doses of amiodarone used in this study did not produce any significant changes in the parameters studied. In some parameters, the variance became larger with time after dosing although no trend in effect was apparent.

Pulmonary effects: *Evaluation of the effects of an acute oral administration of SR33589B on respiratory parameters in unrestrained conscious guinea pigs using whole body plethysmography*. ***RCV0067*** Single oral doses of 10, 30 and 100 mg/kg dronedarone were given to 8 male Duncan-Hartley guinea pigs per group. Theophylline was the positive control. Animals remained in the plethysmograph for 4 hours after receiving drug. Respiratory parameters were obtained pre-drug, 15, 30, 45, 60 , 75, 90, 105, 120, 180 and 240 minutes after dosing. Venous blood was obtained at unspecified times from additional guinea pigs to determine plasma level drug concentration.

Plasma concentrations of drug and metabolite (ng/ml) at Tmax (120 minutes)

	10 mg/kg	30 mg/kg	100 mg/kg
SR33589	6.8	77.3	229.1
SR35021	1.1	13.3	68.4

The positive control produced robust responses on respiratory rate, minute volume, inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow and enhanced pause.
At the highest dose, dronedarone produced a very mild decrease in respiratory rate from 30 to 105 minutes. Tidal volume showed a great deal of variability. However, the MD and HD produced depression of tidal volume as did theophylline from 30 - 60minutes.
Dronedarone produced no discernible effects on minute volume, inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow, and enhanced pause.

Effect of SR33589B on the cardiovascular and respiratory functions in anesthetized dogs after intravenous administration. ***CVR0119***
An intravenous dose of 1-5 mg/kg was given in acetic/acetate buffer to 5 mongrel dogs per sex per group. Hemodynamics, myocardial function and respiratory system were assessed after single intravenous administration of drug to pentobarbital-anesthetized dogs. Hemodynamic, cardiac and respiratory parameters were recorded before administration and every 5 minutes from the 10^{th} to the 40^{th} minute then every 10 minutes to the end of the experiment (90 minutes). QTc was calculated according to Bazett's formula. Results were calculated relative to the control values.

There were significant effects on essentially every parameter.

parameter	1 mg/kg	5 mg/kg
Mean blood Pressure (mm Hg)	slight ↓ from the 50th minute reaching -12 mmHg by 90 minutes	Sudden transient↓ at 10 minutes(-28mm Hg) followed by return to baseline at 20 mins. Progressive ↓ from 40th to 90th minutes(-32mm). Most changes significant.
Heart rate (bpm)	unchanged	↓ from the 10th minute (- 17 bpm) until the end of study(-29bpm) Statistically significant from 50 mins
Cardiac output Ml/min	Moderate increase from 10th-50th minute(+30% on average)Signif at $p<0.05$ from 30-50 minutes.	↑from 10th min(+35%) until 40th min (+20%). ↓ after 70th min(-17% at end of experiment). Statistically significant from 10-20 minutes.
LV SV (ml)	Varied parallel to the changes in CO $P<0.05$ from 30-50 minutes	Statistically signif. ↑ from 10(51%) to 40 min (+35%). Return to baseline at 60 min.
LV pressure (mm Hg)	No signif. changes	Variations parallel to those in mean blood pressure. Signif ($p<0.01$) from 60-90 mins
LV contractility (Mm Hg/s)	↑(+10% on average) from 10-35 mins. ↑(+20% on average) from 40-70 mins.	↓ at 10 mins (-34%, NS) and approaching baseline at 30 mins. Progressive decrease after 50 mins of +35% on average from the 70th minute.
LV work (kg m/min)	↑from 10-50 minutes of +30% on average.	↑ from 15-35 minutes (max of 32% at 20 minutes). Progressive decrease after 50 mins, reaching -36%($p<0.05$) at 90 minutes.
Limb peripheral resistance (mm Hg)	No change	Sudden, transient ↓ of -22mm Hg at 10 minutes then return to baseline at 30 mins. A second ↓ after the 60th minute of 15 -20 mm Hg
Total peripheral resistance (dyn.s.cm-5)	↓ of -20% on average between 10-60 minutes then return to baseline	↓ from 10 mins (-43%, $p<0.001$) to 90 mins (-24%, $p<0.05$) Changes were significant throughout
ECG	Average +10% ↑ in PR interval primarily in the first 40 minutes.	↑RR interval throughout, progressive ↑ again from 60 minutes ($p<0.05$) ↑PR interval from 10-50 minutes (+10-+16% until 25 minutes) approaching baseline by 90 minutes ↑ QT interval at all points

QTc Interval by Bazette's formula (mean±SEM)

timepoint	Dronedarone mg/kg	
	1	5
Baseline	12.48±0.463	12.87±0.318
10	12.03±0.250	12.91±0.139
15	11.92±0.251	13.07±0.196
20	12.24±0.210	13.28±0.231
25	12.30±0.262	13.32±0.191
30	12.13±0.194	13.09±0.293
35	12.46±0.315	13.14±0.316
40	12.05±0.123	13.38±0.320
50	12.17±0.284	13.20±0.330
60	11.87±0.303	13.61±0.437
70	11.95±0.292	13.72±0.445
80	12.15±0.350	13.63±0.288
90	12.29±0.413	13.57±0.338

Summary of respiratory effects

parameter	1 mg/kg	5 mg/kg
Respiratory frequency (cycles/min)	↑ throughout with a maximum +9 cycles per min at 60 mins. Stat signif from 20-60 mins.($p<0.05$)	↑ from 10 minutes (+12 cycles/min, NS) to end (maximum +15 cycles/min at 35 mins, $p<0.05$) Approach towards baseline by 90 mins.
Respiratory flow (l/min)	↑ parallel to that in respiratory frequency Statistically signif ↑ from 30-90 mins. with max +89% at 60 mins	↑ parallel to that in respiratory frequency with a max of +72% at 30 mins. Significant from 30-50 mins)
Tidal volume (ml/cycle)	↓from 10 minutes -30 by ~ 13% ↑from 70-90minutes of ~10%	No change. Very variable measurements.

The single intravenous dose of 5 mg/kg caused a biphasic decrease in mean blood pressure and left ventricular contractility, a negative chronotropic effect and an increase in PR interval lasting 60-90 minutes after dosing. The lower dose of 1 mg/kg caused similar effects of a lesser magnitude.

Effect of SR33589B on the cardiovascular and respiratory functions in anesthetized dogs after intraduodenal administration. ***CVR0125***

Ten mongrel dogs (4 males and 6 females) were anesthetized with pentobarbital. Doses of 12.5 and 25 mg/kg were given via the intraduodenal route. Two different batches of the test article were given to 2 different sets of dogs (n=3 per group). Four dogs were given the high dose. The different parameters were recorded before dosing and after dosing at intervals of 5 minutes from the 10^{th} to the 30^{th} minute then at intervals of 10 minutes until the end of the experiment (150 minutes).

Summary of Cardiovascular Effects

parameter	12.5 mg/kg	25 mg/kg
Mean blood pressure (mm Hg)	↓ from 110 mins(-7mm Hg, p<0.05) thru end of study (-12 mm Hg, p<0.01)	Progressive ↓ from 70 mins reaching -13mm Hg at 110mins(p<0.05) persisting to end
Heart rate (bpm)	↑ from 30 mins (+11 bpm) to end (NS)	Variations from 30-70 mins (+12 bpm, NS) and from 110-130 (-12 bpm, NS)
Cardiac output (ml/min)	↑ from 25 mins(+13%, p<0.05) to 110 mins(+19, p<0.05). Max effect at 70 mins (+31%, p<0.001)	30-70mins avg ↑ of 30% (p<0.05-0.01). Max effect at 60 mins(+36%, p<0.01). Trend back to baseline from 110 mins.
LV stroke volume (ml)	Changes (NS) parallel to CO changes	↑20% from 10-70 mins. Max change at 60 mins (+27%, p<0.001) trend back to baseline
LV pressure (mm Hg)	Progressive ↓ from 110 mins to 150 mins, reaching -15 mmHg (p<0.01)	↑ from 10-50 mins (3-9 mm Hg) ↓from 100 min to end (-19 mm Hg, NS)
LV contractility (mmHg/s)	↑ from 30-70 mins (+14% , NS to max of +20%NS. Slight decrease below baseline after 120 mins.	↑ from 10-100 mins (10-11%). Maximum change at 50 mins(+33%). After 120 mins, values below baseline(-10%,NS)
LV work (kg m/min)	↑ from 25 mins(15%, p<0.05) to 90mins(17%, p<0.05). Max change at 70 mins (31%,p<0.001)	↑ from 10-70mins, signif from 30 mins(31%, p<0.05) to 60 mins (35%, p<0.01). Trending below basleine from 110 to end.
Limb peripheral resistance (mmHg)	No change	Avg ↓ of 10 mmHg from 50-130 mins (p<0.05). Max change at 90 mins (15 mm Hg, p<0.001)
Total peripheral resistance (dyn.s.cm-5)	Values below baseline within 10% except for 70 mins (16%, NS)	↓ from 10 mins (16%, p<0.05) to 130 mins (15%, p<0.05) Max change at 60 mins (-24%, p<0.01)
ECG	Increased HR	Increased HR

Respiratory Parameter Results

parameter	12.5 mg/kg	25 mg/kg
Respiratory frequency Cycles/min	↑ from 80 mins to end (≤4 cycles/min)	↑ from 10 mins (9cycles /min, $p<0.05$) to end of study (14 cycles/min, $p<0.01$ at 150 mins)
Respiratory flow (l/min)	↑ from 20 mins, signif from 70 mins (38%, $p<0.05$) to end of study(+74%,$p<0.01$)	↑ parallel to resp freq:+51%, $p<0.05$ at 10 mins to 127%, $p<0.001$ at 150 mins
Tidal volume(ml/cycle)	Individual variability	Values decreased 10-19% from 80 to 140 mins. NS

After a single intraduodenal administration there was a dose-related increase in cardiac output and a slight positive inotropic effect. Maximum effects tended to be noted 60-70 minutes after dosing. Decreased blood pressure was reported. Consistent with other studies, increased respiratory frequency and flow were also noted.

Renal effects:

Effect of SR33589B on the hydroelectric balance in rats after intravenous administration ***RS0040931005/ION0293***. 1,3 and 10 mg/kg IV given to OFA rats (10/sex/group) .

Summary of effects

parameter	males	females
Specific gravity	No effect	Dose-related increase 1.020-1.025 sig $p<0.1\%$
K+ concentration	↑ 68mM control; 75-73 mM	↑38mM control; 39-61 mM $p<0.1\%$
K+ quantity	No change	↑ 28% (MD) and 46% (HD)
Creatinine conc (mM)	No change	Dose-related ↑; 1.6control, 1.7-2.7 $P<0.1\%$
Creatinine quantity	No change	↑36%(MD), 50%(HD)
Creat Clearance ml/16h	↓at HD 11.5%	↑ at MD 28%, HD 33%
Blood in the urine	"moderate" at MD (9/10 rats) HD 6/10 "large", 4/10"moderate"	LD: 1/10 trace, 3/10 small MD: "Moderate" 10/10 rats HD: 2/10 moderate 8/10 large

Effect of SR33589B on the hydroelectric balance in rats after oral administration ***ION0217*** 10, 30 and 100 mg/kg was given to OFA rats (10/sex/group) as a single oral dose. Both sexes showed a slight, dose-related increase in specific gravity of questionable biological significance. There was a slight increase in excreted creatinine in the HD groups of both sexes. The quantity of excreted potassium decreased at the HD of both sexes. Creatinine clearance was decreased in both sexes. Correspondingly, the concentration of creatinine in the blood increased significantly in both sexes. Serum sodium and potassium increased only the females.
Unlike the intravenous administration study, blood was not reported in the urine under the conditions of this study.

Urinary parameters affected

parameters	males	females
16-hour urine vol(ml)	↓HD(26%) p<0.2%	↓HD(26%) p<0.1%
SG		
Proteins		
Ketone bodies		
Creatinine (mM)	↑at MD(11%), HD(19%) NS	↑LD(13%), MD(11%), HD(19%)
Potassium mM		↑10% at the HD
Potassium µmoles	↓HD(26%) p<1.2%	
Creatinine clearance ml/16hr	↓LD(1%),MD(6%), HD(26%) p<0.1%	↓LD(8%), MD(25%), HD(34%) p<0.1%

Effects of dronedarone on renal function in conscious normotensive rats following repeated oral administration ***PGD0133*** *January 2005*

Doses used were 0 (n=5 per sex, methylcellulose), 10 (n=3 per sex) and 30 mg/kg (n=5 per sex). Dronedarone was given orally to normotensive rats with surgically implanted renal blood flow probes. The animals were dosed once a day for 2 weeks. They were placed in metabolism cages for urine sampling after being water loaded (2% of body weight).
Renal blood flow was measured Days 0, 1,2,3,6,8,9,10,13 and 15. However, results from days 10 and 13 were not reported. Urine was collected days 0,7 and 14. The limited number of animals per group is due to 1)blood flow signals of poor quality and 2) several animals destroyed the external wire of the flow probe.
The results for essentially all parameters do not show any strong or clear patterns. At the same time, there is a great deal of variability, the groups are at very different starting points, and it would be easy to miss a subtle signal.

Gastrointestinal effects:

Effect of SR33589B on intestinal transit in mice after oral administration ***GIT0029***

Doses of 0(gum Arabic aqueous solution) 100, 300 and 1000 mg/kg were given orally to OF1 mice (10M/group). The effect of the drug on intestinal transit was assessed by measuring the length of intestine traveled by a charcoal meal in a 30 minute time period.

The low dose and the highest dose caused decreases in intestinal transit. The effect at the HD was statistically significant.

Effect of administration of SR33589B on intestinal transit in mice (mean±SEM)

group	Dose (mg/kg)	Length of intestine traveled by charcoal (%)	Variation [(a)](%)
Control	0	60±4	
SR33589B	100	69±2 (b)	+14
Control	0	63±4	
SR33589B	300	62±3	-1
Control	0	50±5	
SR33589B	1000(c)	68±4*	+36

(a)in relation to controls

(a) mean ±sem on 9 animals due to technical problem

(b) sedation;charcoal meal very diluted and in low quantity in the intestine

*p≤0.05

Effect of SR33589B on gastric emptying in rats after oral administration. ***GIT0030***

Doses of 0 (gum Arabic), 100 and 300 mg/kg were given orally to OFA rats (10F/group). Sixty minutes after receiving the drug, the effect on gastric clearance of a marker (phenol red) given in aqueous solution. Rats were euthanized 10 minutes after receiving the marker.

Doses of 0 (gum Arabic), 100 and 300 mg/kg were given orally to OFA rats (10F/group). Sixty minutes after receiving the drug, the effect on gastric clearance of a marker (phenol red) given in aqueous solution. Rats were euthanized 10 minutes after receiving the marker. Gastric emptying was evaluated as the difference in the total amount of phenol red administered and the amount remaining in the stomach after 10 minutes.

Effect of oral administration of SR33589B on gastric emptying in rats (mean±sem)

group	Dose (mg/kg)	Amount of phenol red emptied from the stomach (µg)	Variation (a) (%)
Control	0	442±28	
SR33589B	100	396±32	-10
Control	0	501±24	
SR33589B	300	234±37**	-53

(a) variation in relation to controls; **p≤0.01

Under the conditions of the study, the drug delayed gastric emptying. This is opposite to the effects in the mouse study in which gastric emptying was more rapid.

Effect of SR3389B on gastric acid secretion in rats after oral administration ***GIT0031***
SR33589B in 10% gum Arabic was given orally to fasted OFA rats. The effect of the test compound on gastric acid secretion was assessed by measuring the volume and pH of acid secretion in pylorus-ligated rats. Sixty minutes after treatment with the drug the animals were anesthetized and the pylorus ligated. The animals were euthanized 1 hour later.
SR33589B increased the volume and acidity of the secretions.

Dose (mg/kg)	Volume of secretion (µl)	pH of secretion	Total acid (µEq)	H+/ml (µEq/ml)
Control 0	279±71	2.66±0.22(b)	10.0±2.4(b)	36.2±5.0(b)
SR33589B 100 (a)	316±38 (+13)	2.39±0.09 (b) (-10)	14.3±2.3(b)	44.2±4.0(b) (+22)

(a) variations (%) in relation to controls
(b) results for 7 animals

While there were no statistically significant results, the one dose of drug caused an increased volume of more acidic secretions. A second dose would have made this study more informative (i.e., is the effect dose-related?).

Abuse liability: not done

Other:

Effects of chronic oral SR33589B administration on plasma thyroid hormone levels in rat. Comparison with amiodarone. ***685-4-013***
Male Wistar rats (7 groups of 10 animals) were dosed with 50, 100 or 150 mg/kg amiodarone or 50, 100 or 150 mg/kg of dronedarone given as an oral suspension in 5% aqueous gum arabic. The rats were dosed once a day for 14 days. T3, T4 and rT3 were measured by RIA kits.

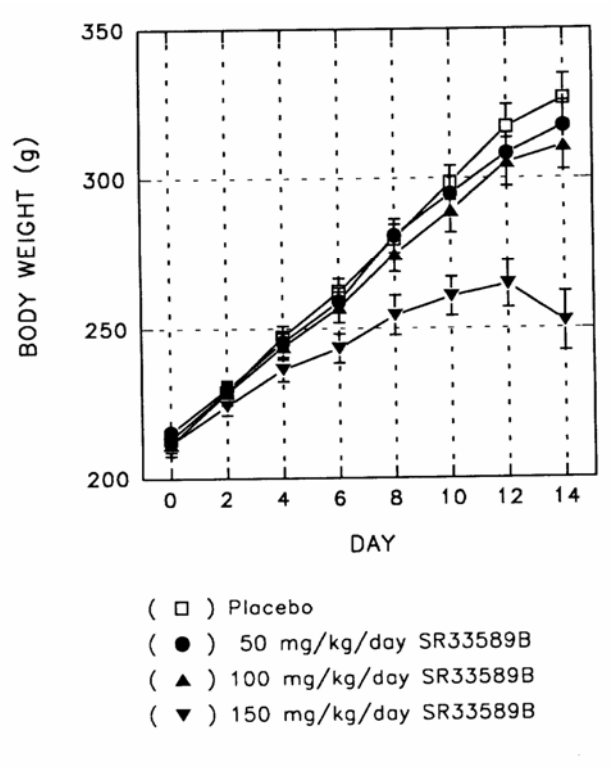
Dronedarone caused a decrease in T4 at the highest dose and a decrease in the T4/T3 ratio. Amiodarone caused a decrease in T4 and T3 and an increase in rT3. The results are shown below in the sponsor's summary.

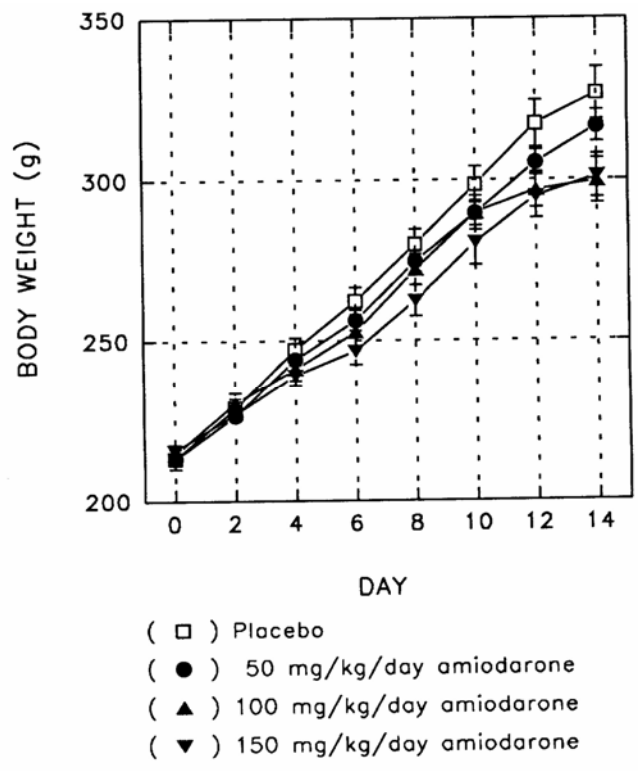
Effects of orally administered dronedarone and amiodarone on body weight

group	Body weight (g)	Product plasma concentrations (nmol/l)
Control	327±25(10)	
SR33589B 50 mg/kg	318±26 (10)	0.17±0.03 (7)[a]
SR33589B 100 mg/kg	311±24 (9)	0.17±0.05(8) [b]
SR33589B 150 mg/kg	252±31*** (10)	0.82±0.37(10)
Amiodarone 50 mg/kg	316±16(10)	0.49±0.17(10)
Amiodarone 100 mg/kg	300±22 (10)	2.05±0.41(10)
Amiodarone 150 mg/kg	301±21(10)	2.58±1.11(10)

**p<0.01, ***p<0.001 compared to control group; (n)number of determinations

[a] No detectable levels of SR33589B in 3 rats (<0.017 nmol/ml)





[b] No detectable levels of SR33589B in 1 rat (<0.017 nmol/ml)

Effects of orally administered dronedarone and amiodarone on thyroid hormone levels

group	T4 (ng/ml)	T3 (ng/ml)	rT3 (pg/ml)	T4/T3 ratio
Control	54±5(10)	0.93±0.10(9)	60±13(9)	58±3(9)
SR33589B 50 mg/kg	56±12(10)	0.98±0.17(10)	61±22(10)	57±6(10)
SR33589B 100 mg/kg	49±9(9)	0.76±0.12(9)	63±17(9)	62±4(8)
SR33589B 150 mg/kg	35±9***(10)	0.76±0.18(10)	48±14(10)	46±6***(10)
Amiodarone 50 mg/kg	62±10(10)	0.94±0.10(8)	133±21***(10)	66±11(8)
Amiodarone 100 mg/kg	61±11(10)	0.81±0.15(10)	210±21***(9)	69±10**(10)
Amiodarone 150 mg/kg	50±9(10)	0.73±0.09**(10)	222±48***(10)	75±2***(10)

**p<0.01, ***p<0.001 compared to control group
(n)number of determinations (in duplicate for T4, T3, rT3 measurements)
[a] No detectable levels of SR33589B in 3 rats (<0.017 nmol/ml)
[b] No detectable levels of SR33589B in 1 rat (<0.017 nmol/ml

The highest dose of dronedarone caused a decrease in body weight gain apparent from the 6th day.

In this study, the highest dose of dronedarone caused a marked decrease in bodyweight gain while the highest dose of amiodarone had little effect on body weight. Amiodarone had minimal effect on T4 levels, caused a decrease in T3, an increase in rT3 and finally an increase in the ratio of T4/T3. It is questionable whether 2 weeks is sufficient time to assess thyroid hormone effects. A question that usually arises is whether there is an absolute effect on the production of hormone or whether there is a change in the time in circulation. The usual argument is that thyroid effects seen in the rat are not relevant to the human due to the differences in half life between the species. A study of 28 days duration using radiolabels to track the effects of the drugs on the biological half-lives of the thyroid hormones would have been preferable. Also, other studies with this drug have shown an endocrine signal in the females. In the interests of investigating that signal and the potential relevance to humans both sexes should have been evaluated.In this study there was minimal detected effect on thyroid hormones except for those induced by treatment intolerance (a sick euthyroid effect?) found at a maximally tolerated dose. However, that is not necessarily a complete description of the thyroid effects of dronedarone when administered over a longer time period.

Phospholipid content of lung and liver in rats treated orally for 14 days with SR3389B and amiodarone ***685-4-040***

Seven groups of 10 male Wistars/group were treated orally for 14 days with doses of 50, 100 and 150 mg/kg/day. Twenty-four hours after the end of treatment the rats were anesthetized and blood and tissues collected. Lipids were extracted from plasma and from tissues (lung and liver)

Again, the high dose of 150 mg/kg/day caused a decrease in body weight apparent from the 6th day. There was however a dose-related decrease in body weight with both drugs.

Effect of dronedarone treatment on body and tissue weight

	vehicle	SR33589B		
		50	100	150
Body weight	326.6±8.1(10)	317.5±8.3(10)	310.8±8.1(9)	252.3±9.9***(10)
Lung/body weight x 100	0.472±0.027(10)	0.478±0.012(10)	0.475±0.016(8)[a]	0.450±0.013(9)[a]
Liver/body weight x 100	4.46±0.13(10)	4.48±0.08(10)	4.09±0.08(9)	3.82±0.09***(10)

Mean ±sem of (n) animals
[a]: lung weight not determined in 1 rat
***:p≤0.001

Effect of amiodarone treatment on body and tissue weight

	vehicle	amiodarone		
		50	100	150
Body weight	326.6±8.1(10)	316.4±4.9(10)	299.6±6.9(10)	301.1±6.7(10)
Lung/body weight x 100	0.472±0.027(10)	0.442±0.011(10)	0.477±0.017(10)	0.499±0.013(10)
Liver/body weight x 100	4.46±0.13(10)	4.42±0.09(10)	4.70±0.09(10)	4.77±0.15(10)

Mean ±sem of (n) animals
[a]: lung weight not determined in 1 rat
***:p≤0.001

The results of the lipid content are shown below. The sponsor's table has been divided for greater clarity.

Effects of orally administered drug on plasma lipids

	SR33589B(mg/kg/day)			
	Vehicle (0)	50	100	150
Proteins mg/ml	61.6±0.07	63.0±0.7	61.8±0.9	61.4±1.3
Phospholipids (nmol/mg/prot)	36.6±1.5	35.1±1.3	35.3±1.6	35.1±1.3
Total cholesterol Nmol/mg prot	11.8±0.7	11.4±0.8	13.8±0.5	12.5±0.6
Free cholesterol Nmol/mg prot	7.5±0.9	8.8±0.8	10.8±0.8	9.3±0.8
Esterified cholesterol nmol/mg prot	4.4±0.8	2.6±0.7	2.9±0.5	3.2±1.1
Total cholesterol/ phospholipids	0.33±0.02	0.33±0.02	0.39±0.02	0.36±0.01
Compound nmol/mg prot		0.0027±0.0002[a]	0.0028±0.0002[b]	0.0136±0.0021

mean±sem
[a] no detectable levels of SR33589B in 5 rats (<0.17 nmol/ml plasma)
[b] no detectable levels of SR33589B in 1 rats (<0.17 nmol/ml plasma)
***p≤0.001

Effects of orally administered drug on plasma lipids

	amiodarone (mg/kg/day)			
	vehicle	50	100	150
Proteins mg/ml	61.6±0.07	63.4±0.6	61.3±0.7	62.9±0.7
Phospholipids (nmol/mg/prot)	36.6±1.5	40.9±1.6	35.1±2.1	41.2±1.9
Total cholesterol Nmol/mg prot	11.8±0.7	14.0±0.7	17.6±0.8***	18.3±1.0***
Free cholesterol Nmol/mg prot	7.5±0.9	8.9±0.5	13.1±0.9***	14.8±0.7***
Esterified cholesterol nmol/mg prot	4.4±0.8	5.2±0.7	4.5±0.7	3.6±1.2
Total cholesterol/ phospholipids	0.33±0.02	0.34±0.02	0.52±0.04***	0.44±0.01***
Compound nmol/mg prot		0.0077±0.0009	0.0337±0.0024	0.0410±0.0058[c]

mean±sem
[c] one value rejected
***p≤0.001

Effects of orally administered drug on tissue lipids

	SR33589B(mg/kg/day)				Amiodarone (mg/kg/day)		
	Vehicle (0)	50	100	150	50	100	150
Proteins mg/ml	207.2 ±2.5	205.5 ±2.7	216.4 ±4.0	223.0 ±5.4	215.2 ±5.6	210.9 ±3.0	218.5 ±2.1**
Phospholipids (nmol/mg/prot)	156.1 ±4.5	151.6 ±4.4	178.2 ±4.5**	162.6 ±4.4	149.9 ±6.0	175.3 ±6.1	187.6 ±3.4***
Total cholesterol Nmol/mg prot	22.92 ±0.84	23.50 ±0.86	23.21 ±0.81	22.9 ±0.91	21.85 ±1.05	22.43 ±0.72	20.71 ±0.89
Total cholesterol/ phospholipids	0.15 ±0.01	0.16 ±0.01	0.13 ±0.01	0.14 ±0.01	0.15 ±0.01	0.12 ±0.01	0.11 ±0.01***
Compound nmol/mg prot		0.0069 ±0.0011[b]	0.0060 ±0.0008[c]	0.1581 ±0.0343[d]	0.0845 ±0.0071	0.197 ±0.0033	0.246 ±0.036

mean±sem
[a] no detectable levels of SR33589B in 5 rats (<0.17 nmol/ml plasma)
[b] no detectable levels of SR33589B in 1 rats (<0.17 nmol/ml plasma)
***p≤0.001

There are small but statistically significant changes in serum phospholipids and cholesterol following amiodarone treatment. These changes are slightly greater than those seen with dronedarone. There is some variability of tissue levels. How sensitive a measure of a subtle effect are these parameters? One test that was not done was histopathology or electron microscopy, which might have been better able to detect low grade or early changes.

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY 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] The tables as provided by the sponsor were of poorly scannable or transferable quality and could not be used without producing a "Compressed File Virus" corruption of this review.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.8 **2.6.4.1 Brief summary** The absorption, distribution, metabolism and excretion of dronedarone was evaluated in CD-1 mice, Sprague-Dawley rats, New Zealand White rabbits, Beagles and Macaca fasicularis monkeys. There was also some examination of the effect of dronedarone on the CYP450 system. Oral administration studies were conducted using a suspension of drug in 0.6% methylcellulose or, for dogs, gelatin capsules. Intravenous administration studies with formulations of PEG400/sodium acetate buffer or mannitol/sodium phosphate were carried out in rats, dogs and macaques.

The oral bioavailability is relatively low: 14-22%. The volume of distribution is large, 12 to 66 l/kg with a moderately high systemic clearance of 2-4 l/h/kg. The apparent terminal half-life after oral dosing is from 2-7 hours.

In all species studied, dronedarone was highly protein bound (>99%). The drug stays primarily in the plasma, not the red blood cells. There is wide tissue distribution (lung, liver, spleen, kidney, myocardium) including crossing the placental barrier and entering milk. Radioactivity has also been identified in the brain, indicating that either drug and/or its metabolites cross the blood brain barrier. Low levels of radioactivity persisted after 336 hours in the liver, spleen and testis. Melanin binding was also demonstrated.

The drug is extensively metabolized and the sponsor's diagram of the proposed metabolic scheme can be found in the conclusion of this section. N-debutylation leads to SR35021, an active metabolite and the main circulating species in mice. A carboxylic acid derivative, SR90154, has limited pharmacologic activity and is one of the main circulating metabolites in rats and dogs.

The main route of excretion after both oral and intravenous administration is via the feces. There is a minor amount excreted in the urine. Aliquots of urine treated with β-glucuronidase indicate that there is little if any conjugation of the metabolites.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

Determination of SR33589 and its N-debutyl metabolite (SR35021) in mouse plasma by HPLC-UV following liquid/liquid extraction. ***DOS0320*** The sponsor determined a linear range of 0.04-1.0 mg/l with a limit of detection 0.020 mg/l and a limit of quantitation of 0.04 mg/l. Within day variability (CV) was from 3.0-6.6% at the LOQ for SR33589 and from 3.0-5.3%. Reproducibility over 5 days (CV) was <20% for both parent and metabolite.

Validation of an electrospray LC-MS/MS detection method for the quantitative analysis of SR33589 and its metabolite SR35021 in mouse plasma following a Disk SPE (96 well plate) extraction. ***DOS0465*** The linear range was determined to be 1 – 2000 ng/ml. The limit of detection was not determined and the limit of quantitation was reported to be 1 ng/ml. The assay variability (CV) was determined to be ≤5% for each of the four standard concentrations of parent drug.

Validation of an LC/MS-MS detection method for the quantitative analysis of SR33589 and its metabolite SR35021 in rat plasma following a Disk SPE (96 well plate) extraction ***DOS0444.*** The linear range of the assay was determined to be 0.5-1000 ng/ml for both SR33589 and SR35021. The limit of detection was not determined. The limit of quantitation was 0.5 ng/ml for both chemicals.

HPLC-UV assay of SR33589 and its N-debutyl metabolite, SR35021, in rat plasma samples ***DOS0150***

Rat plasma collected with sodium heparin was spiked with 0.125 μg of SR34193 (internal standard) and the test compounds, water/methanol and sodium acetate buffer. After vortexing the mixture was allowed to stand on the extraction column. SR33589, SR35021 and the internal standard were eluted, dried under nitrogen and re-solubilised. Aliquots were injected onto the HPLC system.

The retention times listed indicate good separation of the peaks:
SR33589- approx 7.3 min
SR35021- ~5.9 min
SR34193~10.8 min

Limit of detection: 0.01 mg/l for SR33589 and SR35021
Limit of quantitation: 0.02mg/l for SR33589 and SR35021

It was also determined that the SR33589 and SR35021 did not bind to glass or plastic material in contact with plasma or water/methanol solution.

The extraction efficiency was calculated at 0.02, 0.2 and 1 mg/l using plasma samples spiked with known amounts of SR35021A and SR33589B and SR34193. The same concentrations were used to determine precision and accuracy of the assay method.

Long term stability of SR33589 and SR35021 in frozen rat plasma. ***SPP0010***
SR33589 plasma standards were prepared at 0.059, 0.197 and 0.690 mg/l concentration levels and SR35021 at 0.059, 0.197 and 0.692 mg/l. Six determinations of SR33589 and SR 35021 were made at each level at 24 months. At each period a daily calibration curve was performed using validated batches of the two chemicals. Concentrations were determined using HPLC-UV analysis. There was a slight decrease in concentration over 24 months for both chemicals.

Mean concentration (SD) of five determinations

SR33589		
theoretical	After 24 months	Variation range (%)
0.059	0.053±0.002	-10.2
0.197	0.194±0.003	-1.5
0.690	0.697±0.039	-1.6
SR35021		
0.059	0.053±0.002	-10.2
0.197	0.193±0.003	-2.0
0.692	0.714±0.030	+3.2

The samples were relatively stable (variation range <20%) when stored at -18ºC in the dark over 24 months.

Long term stability of SR33589 and SR35021 in frozen dog plasma ***SPP0011***
SR33589 and SR35021 plasma standards were prepared at 0.050 and 0.400 mg/ml concentration levels. Five determinations of SR33589 and SR35021 were made at each level at 3 and 16 months. At each period a daily calibration curve was performed using validated batches of SR33589 and SR35021. Concentrations were determined by HPLC-UV detection.

Mean concentration (SD) for 5 determinations

SR33589		
theoretical	After 16 months	Variation Range%
0.050	0.049(0.002)	-2.0
0.400	0.407(0.015)	+1.8
SR35021		
0.050	0.041(0.001)	-18.0
0.400	0.370(0.012)	-7.5

The samples were relatively stable (variation range <20%) when stored at -18ºC in the dark for 16 months.

Radioachemical stability and binding to glass and plastic of 14C labeled 33589B in vehicles used for animal studies. ***SFA0002***

Male and female Sprague-Dawley rats used for excretion balance and distribution studies were given 30 mg/kg p.o. (in methylcellulose) or 10 mg/kg i.v. (PEG400/sodium acetate) of SR33589B. The radioactivity administered was 1.11 MBq (30 μCi/kg) for both sexes and routes of administration. The radiochemical purity was determined to be 99.9%.

Radiochemical stability was assessed over 48 hours after preparation and storage either at room temperature or at 4ºC and reported as "good".

The sponsor provided a summary of the concentrations of 14C labeled SR33589B dissolved in formulations as a function of the sampling time. This is reproduced below.

Transfer number	Time (minutes)	Oral formulation		Intravenous formulation	
		Glass	plastic	Glass	plastic
0	0	237.26	244.64	222.18	217.13
1	10	232.83	227.99	211.24	207.51
2	20	212.60	248.82	215.69	219.52
3	30	224.61	217.20	208.62	219.23
4	40	228.88	235.91	214.09	207.34
5	50	212.78	222.90	216.63	213.22
6	60	222.10	226.99	213.26	217.10
At t=60, after last transfer, binding to container accounted for(% of what not defined)		6.39%	7.22%	4.01%	0.01%

2.6.4.3 Absorption

Pharmacokinetics and absolute bioavailability of SR33589B following single 10 mg/kg intravenous and 30 mg/kg oral administration of (carbonyl-14C)-SR33589B to male and female Sprague-Dawley rats. ***Abs0102***

Three animals/sex/sampling time/route. Radioactivity: $1.1x10^3$ KBq/kg
Sampling times:

IV: 0, 0.083, 0.05,1,2,3,4,5,6,8,10, 24, 32, 48 and 72 hours
PO: 0,0.5, 1, 1.5, 2,3,4,6,8, 10, 24, 32, 48 and 72 hours.

Plasma levels of SR33589B and SR35021 were determined.

Pharmacokinetic Summary

	intravenous		oral	
parameters	male	female	male	Female
Co(mg/l)	0.72	1.03		
Cmax(mg/l)			0.19	0.18
Tmax(hour)			4.0	4.0
$T_{1/2}$(h)	6.1	12.5	6.4	6.6
$AUC_{0\text{-}10h}$ (mg.h/l)	1.74	1.75	1.15	0.96
$AUC_{0\text{-}inf}$ (mg.hr/l)	2.28	2.64		
Cl(l/h.kg)	4.4	2.3		
Vd(l/kg)	38.5	66.2		
F%			22	18

The concentrations of SR35021 were very low. Only Cmax and Tmax were determined.

Mean(SD) values for SR35021

	PO		IV	
	males	females	males	females
Cmax (mg/l)	0.052(0.046)	0.030(0.030)	<0.020	<0.020
Tmax (h)	4.0	4.0		

Pharmacokinetic profiles of SR33589B and SR35021 following a single (30 mg/kg) oral or (5 mg/kg) intravenous infusion administration of SR33589B or SR35021A to Beagles. ***Abs0209***

Two dogs per sex, approximately 2 years of age were used.
Sampling times:

IV: 0.083, 0.25, 0.5, 1,2,3,4,6,8,10,24,32,48,54,72 and 96 hours post-dose
PO: 0.5,1,1.5,2,3,4,6,8,10,24, 32,48, 54, 72 and 96 hours post-dose.

Plasma level determination of parent drug and SR35021 was assessed by HPLC-UV.

Summary of pharmacokinetic parameters

	SR33589B		SR35021	
	Intravenous	oral	Intravenous	oral
$AUC_{0\text{-}Clast}$ (mg.h/l)	2.309±1.278	1.672±2.810	0.614±0.215	0.181±0.028
$AUC_{0\text{-}inf}$ (mg.h/l)	2.50±1.287	3.363±3.719*	0.713±0.235	NC
$Cl_{0\text{-}inf}$ (l/h.kg)	2.374±1.005		7.628±2.479	
$Cl_{0\text{-}inf}$ (l/h)	29.421±12.367		92.038±30.757	
Vd(l/kg)	12.016±5.591		23.658±7.653	
$T_{1/2}\beta$(h)	3.4±0.7	3.8±0.0*	2.2±0.3	
$MRT_{(0\text{-}inf)}$(h)	3.570±0.973		2.426±0.421	
tmax		3.55±1.94		1.70±0.56
Cmax(mg/l)		0.241±0.349		0.071±0.043
F%		14±13*		

*two values

After oral administration, SR35021 was quantifiable in only 1 animal due to limitations of assay sensitivity. There was substantial inter-animal variability in the plasma concentrations and subsequent pharmacokinetic parameters. Both parent drug and the active metabolite showed a large volume of distribution as well as plasma clearance.

The pharmacokinetics of SR33589 following a single 1 mg/kg intravenous administration of (carbonyl-14C)-SR 33589B to male Macaca [sic] monkeys. ***ABS0103***

Four males were given 1.85 $x10^3$ KBq/kg.
Sampling times: 0.083, 0.25, 0.5, 1,2,3,4,6,8,10,24, 48, 120 and 168 hours.

SR35021 was not detected.

SR33589B PK parameters (mean ±SD)

Co(mg/l)	0.163(0.045)
T1/2(h)	1.94(0.49)
AUCobs(mg.h/l)	0.154(0.061)
AUC 0-inf (mg.h/l)	0.233(0.085)
Cl(l/h.kg)	4.92(2.35)
Vd(l/kg)	12.81(3.45)

2.6.4.4 Distribution

Quantitative tissue distribution and excretion balance of (carbonyl-14C)SR33589B following single oral administration (30 mg/kg) to the male Sprague-Dawley rat. ***DIS0045***

Eighteen males received drug in a methylcellulose suspension containing 0.37MBq/ml. The tissues of 3 animals per time point were analyzed at 1,4,8,24,96 and 168 hours post-dose.

The highest concentration of radioactivity was reported for the small intestinal contents followed by the large intestinal contents. After that, the liver showed the greatest concentration.

TABLE 4 : MEAN TISSUE CONCENTRATIONS OF THE RADIOACTIVITY OBSERVED AFTER SINGLE 30 mg.kg^{-1} ORAL ADMINISTRATION OF (carbonyl-^{14}C)SR 33589B TO MALE SPRAGUE-DAWLEY RATS.

(Results expressed as mg Eq.SR 33589B.kg^{-1} of tissue and normalized to a theoretical dose of 30 mg.kg^{-1}).

TIME (h)	MEAN 1	MEAN 4	MEAN 8	MEAN 24	MEAN 96	MEAN 168
WEIGHT(g)	269	268	259	263	264	261
ADM.DOSE (mg.kg^{-1})	30.30	30.55	29.71	30.24	30.04	30.00
BRAIN	0.15	0.23	0.15	0.058	0.10	ILQ
LUNGS	22	47	20	0.44	0.091	ILQ
LIVER	51	39	8.4	1.4	0.40	0.12
HEART	7.5	9.0	2.6	0.15	0.068	ILQ
KIDNEYS	14	18	6.5	0.46	0.17	ILQ
TESTICLES	0.16	0.65	0.63	0.40	0.31	0.17
OESOPHAG.	18	18	2.5	0.28	0.21	ILQ
STOMACH	79	27	2.2	0.13	0.051	ILQ
SML INT.	121	83	11	0.43	0.15	ILQ
CAECUM	2.6	18	26	0.90	0.095	ILQ
COLON	2.3	11	14	0.63	0.11	ILQ
RECTUM	2.0	5.4	38	0.61	0.12	ILQ
FLAT BONE	1.5	3.1	1.2	0.36	0.69	ILQ
BONE SURF	1.3	2.6	0.91	0.22	0.35	ILQ
SALIV G.	5.1	12	6.3	0.082	ILQ	ILQ
THYMUS	1.7	5.6	4.2	0.25	ILQ	ILQ
PANCREAS	7.5	17	7.9	0.52	ILQ	ILQ
SPLEEN	9.4	14	5.0	0.19	ILQ	ILQ
PROSTATE	2.0	6.0	5.5	0.19	ILQ	ILQ
BLADDER	2.7	5.1**	5.1	ILQ	ILQ	ILQ
EYES	0.52	1.0	0.47	ILQ	ILQ	ILQ
THYROID	9.8	12	3.6	ILQ	ILQ	ILQ
ADRENALS	16	28	11	ILQ	ILQ	ILQ
SPINAL C.	0.16	0.29	0.30	ILQ	ILQ	ILQ
B. MARROW	4.5	11	4.6	ILQ	ILQ	ILQ
HYPOPHYS.	4.0	20	12	2.2	ILQ	ILQ
HARDER GL	30	8.4	10	4.3	1.1	0.40
MUSCLE	2.9	5.1	2.1	ILQ	ILQ	ILQ
WHITE FAT	2.1	5.8	3.8	0.20	ILQ	ILQ
BROWN FAT	8.7	14	3.1	0.17	ILQ	ILQ
SKIN	1.6**	3.3	1.8	0.13	ILQ	ILQ
PLASMA	1.4	1.9	0.39	0.022	ILQ	ILQ
TOT.BLOOD	1.1	1.5	0.30	ILQ	ILQ	ILQ
GAST.CT	42	7.9	0.88	0.032	ILQ	ILQ
S.INT.CT	229	200	55*	1.6	0.13	0.017
L.INT.CT	0.12	75	85*	1.9	0.069	ILQ
CARCASS	1.8	4.2	2.3	0.17	0.090	ILQ

Summary of radioactivity at specific time points

organ	Mg Eq SR33589B.kg^{-1} of tissue		Ct 1/c pl	$T_{1/2}$ (h)
	Ct1	Ct2		
Lungs	22	ILQ	15	31
Liver	51	0.12	35	41
Kidneys	14	ILQ	9.4	50
plasma	1.4	ILQ	1.0	4

Ct1 = 1 hr after dosing

Ct2=168 hr after dosing=last measurement

Cpl= plasma concentration 1 hour after administration

The maximum concentration in most tissues was seen at 4 hours after dosing except for the liver, stomach and contents, small intestine and contents, and Harderian glands. The named tissues showed maximum concentration at the first sampling point of 1 hour. Cecum, colon, rectum and associated contents showed maximum concentration at 8 hours post-dose. At 96 hours post-dose the highest concentration was reported for the "flat bone."

Tissue distribution after a single (30 mg/kg) oral administration of (^{14}C-carbonyl) SR335589B to the female Sprague-Dawley rat ***DIS0198***

Seven female rats, 1 animal/time point, were given a single dose of drug as a solution in methyl cellulose containing 3.70 MBq/kg. Sampling times were 1,4,8,24,96, 168 and 336 hours post-dosing.

The text of the report states that highest radioactivity levels were observed in gut content. This was not apparent in the data presented. It can be said that the radiolabeled drug was rapidly distributed to the tissues sampled and was cleared by 168 hours after dosing. Liver, spleen, lung and kidney showed the highest levels of drug-associated label.

. Quantitative tissue distribution by Liquid Scintillation Counting : tissue concentrations expressed as mg Eq/kg of tissue

SAMPLING TIMES (hours)	1	4	8	24	96	168	336
Blood	0.624	0.678	1.141	UDL	UDL	UDL	UDL
Plasma	0.651	0.719	1.087	0.129	UDL	UDL	UDL
Brain	0.140	0.132	0.309	UDL	UDL	UDL	UDL
Kidney	4.502	8.056	15.945	0.464	0.089	UDL	UDL
Liver	15.007	15.829	20.091	1.466	0.175	0.101	UDL
Lung	2.509	12.231	22.136	0.735	UDL	UDL	UDL
Muscle	0.727	1.710	5.171	0.189	UDL	UDL	UDL
Myocardium	1.611	3.637	6.149	0.188	UDL	UDL	UDL
Spleen	5.113	21.346	20.643	0.629	UDL	UDL	UDL
Thymus	0.782	2.195	7.772	0.529	UDL	UDL	UDL
White fat	3.184	*	6.895	0.366	UDL	UDL	UDL

* technical problem
UDL = under detection limit

. Half live estimation

No half-life value was determined

Tissue distribution of radioactivity following a single (30 mg/kg) oral administration of (Carbonyl-^{14}C) SR33589B to the pregnant Sprague-Dawley rat ***DIS0231***

Pregnant rats (3/sampling time/day of gestation) were used. The drug was suspended in methyl cellulose with 0.1398 MBq/mg. Sampling times were 2,6, 24 and 48 hours for blood, plasma and tissues at day 9 and day 18 of gestation. Analysis was by quantitative whole body radioluminography (QWBRLG) and liquid scintillation counting (LSC).

Gestation Day 9: Drug was rapidly distributed to all tissues analyzed. There was an increase in radiolabel in the embryo from 2 to 6 hours. Drug-associated label was reported for the ovaries, mammary gland and uterus also. The sponsor's results are shown below.

. Mean tissue concentrations on day 9 of gestation (results expressed as mg Eq. non salified compound/ (n=3 animals /sampling time except when indicated between brackets).

Sample times	2 hours		6 hours		24 hours		48 hours	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Blood	1.557 (*)	0.435	1.542 (*)	0.338	ND	NC	ND	NC
Brain	UDL	NC	UDL	NC	ND	NC	ND	NC
Harder's gland	3.100	0.728	15.667	2.122	6.704	1.343	1.677	0.299
Kidney	15.037	4.143	22.490	4.494	1.308 (*) (n=2)	NC	ND	NC
Liver	41.920	12.099	35.305	7.366	2.771	0.321	0.667 (*)	0.054
Lung	14.476	4.874	36.097	3.165	1.000 (*)	0.273	ND	NC
Mammary gland	4.030	1.747	10.061	1.083	ND	NC	ND	NC
Myocardium	7.840	2.258	11.899	2.449	ND	NC	ND	NC
Ovaries	6.251	2.479	9.476	2.217	1.066 (*) (n=1)	NC	ND	NC
Plasma [1]	0.940	0.199	1.107	0.338	0.163	0.103	UDL	NC
Spleen	19.140	5.060	35.430	6.754	1.369 (*)	0.272	ND	NC
Thymus	2.692	0.884	10.980	1.883	1.016 (*)	0.328	ND	NC
Uterus	2.662	0.298	6.738	1.102	1.057 (*) (n=1)	NC	ND	NC
Embryo	4.659	1.065	10.148	1.398	0.941 (*) (n=1)	NC	ND	NC

UDL = Under detection Limit LOD = 0.495 mg Eq./kg
(*) = Under Quantification Limit LOQ = 1.650 mg Eq./kg
NC = Not calculated
ND = Not determined (No measured data because under the background noise of the detector system)
[1] = values from LSC counting

The data for gestation day 18 show similar results

Sample time	2 hours		6 hours		24 hours		48 hours	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Blood	2.844	0.270	2.503	0.496	ND	NC	ND	NC
Brain	UDL	NC	0.611 (n=2) (*)	NC	UDL	NC	ND	NC
Harder's gl.	7.472	1.448	21.636	3.588	7.900	0.246	3.523	1.076
Kidney	27.017	2.160	29.642	6.389	1.329 (*)	0.182	ND	NC
Liver	86.207	4.212	47.073	12.695	2.184	0.131	1.028 (*)	0.166
Lung	34.649	1.898	59.396	7.950	1.812	0.268	ND	NC
Mammary gl.	8.751	0.644	18.592	2.088	1.768	0.071	0.703 (n=2) (*)	NC
Myocardium	16.627	0.837	14.922	2.944	ND	NC	ND	NC
Ovaries	17.575	0.646	12.126	3.476	0.563 (n=2) (*)	NC	ND	NC
Plasma [1]	1.657	0.023	1.120 (n=2)	NC	0.080 (n=2)	NC	UDL	NC
Spleen	33.253	3.824	45.214	6.059	1.642 (*)	0.194	ND	NC
Thymus	7.242	0.612	16.753	2.237	2.312	0.469	ND	NC
Placenta	10.041	0.393	9.544	2.870	0.623 (n=2) (*)	NC	ND	NC
Decidua	7.815	1.904	21.950	2.652	8.617	0.721	6.532	NC
Trophoblast	12.492	0.854	17.692	0.428	3.562	0.497	2.819 (n=2)	NC
Fetus	UDL	NC	0.534 (n=1) (*)	NC	UDL	NC	ND	NC
Fetus liver	0.888 (*)	0.017	1.546 (*)	0.199	UDL	NC	ND	NC

UDL = Under detection Limit LOD = 0.495 mg Eq./kg
(*) = Under Quantification Limit LOQ = 1.650 mg Eq./kg
NC = Not calculated
ND = Not determined (No measured data because under the background noise of the detector system)
[1] = values from LSC counting

After a single oral dose of [carbonyl-^{14}C]SR33589B to pregnant Sprague-Dawley rats, detectable transfer of drug-associated radioactivity to the embryo was demonstrated. There was also significant uptake of radioactivity by smooth muscle – containing structures (uterine wall and decidua) and by the trophoblastic area (area of exchange between fetus and female).

Table (5.1) 4 - Mean tissue to maternal plasma ratios after a single (30 mg/kg expressed as non salified compound) oral administration of [Carbonyl-^{14}C] SR33589B to the pregnant Sprague-Dawley rat on day 18 of gestation
(n = 3 animals /sampling time except when indicated between brackets)

Sample time	2 hours		6 hours		24 hours		48 hours	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Blood	1.713	0.143	1.823 (n=2)	NC	NC	NC	NC	NC
Brain	NC	NC	0.663 (n=2)	NC	NC	NC	NC	NC
Decidua	4.688	1.099	21.848 (n=2)	NC	113.145 (n=2)	NC	NC	NC
Fetus	NC	NC	0.417 (n=2)	NC	NC	NC	NC	NC
Fetus liver	0.537	0.017	1.425 (n=2)	NC	NC	NC	NC	NC
Harder's gl.	4.488	0.823	21.850 (n=2)	NC	108.888 (n=2)	NC	NC	NC
Kidney	16.278	1.091	21.456 (n=2)	NC	20.248 (n=2)	NC	NC	NC
Liver	52.028	2.313	31.167 (n=2)	NC	30.971 (n=2)	NC	NC	NC
Lung	20.907	1.017	50.430 (n=2)	NC	27.952 (n=2)	NC	NC	NC
Mammary gl.	5.274	0.326	17.547 (n=2)	NC	24.600 (n=2)	NC	NC	NC
Myocardium	10.026	0.369	11.078 (n=2)	NC	NC	NC	NC	NC
Ovaries	10.602	0.260	8.011 (n=2)	NC	9.917 (n=2)	NC	NC	NC
Placenta	6.067	0.293	1.000 (n=2)	NC	1.000 (n=2)	NC	NC	NC
Plasma	1.000	NC	6.102 (n=2)	NC	8.192 (n=2)	NC	NC	NC
Spleen	20.015	2.056	36.817 (n=2)	NC	23.006 (n=2)	NC	NC	NC
Thymus	4.363	0.313	16.194 (n=2)	NC	35.490 (n=2)	NC	NC	NC
Trophoblast	7.538	0.476	15.733 (n=2)	NC	53.529 (n=2)	NC	NC	NC

Investigation of possible affinity of total radioactivity in melanin containing tissue following a single oral (30 mg/kg) administration of [^{14}C-carbonyl]-SR33589B to pigmented rats. ***DIS0407***

Male Lister Hooded rats, 3 per timepoint, received drug in a methylcellulose suspension with 3.49µCi/mg of radiolabel. Eye, liver, uveal tract and skin (pigmented and non-pigmented) were sampled at 24, 96 and 336 hours after dose administration. Analysis was by QWB autoradiography.

The highest concentrations were observed at 24 hours for all tissues assessed. The uveal tract showed the greatest levels of radioactivity. Unfortunately the retinal pigment epithelium was not also analyzed (or reported). Pigmented skin showed detectable levels of radio-label while there were undetectable amounts in the non-pigmented skin. Overall the data is suggestive that the drug binds to melanin.

Summary of radioactivity in melanin-containing tissue

	Timepoint (ng Eq unsalified compound/g ±SEM)		
	24 h	96h	336h
Eye	14530±6940	3405±1716	2299±519
Liver	1428±209	94	BLQ
Skin(non-pigmented)	BLQ	BLQ	BLQ
Skin (pigmented)	1306±125	1229±238	BLQ
Uveal tract	49840±9676	18501±9289	12590±3523
LOQ	340±82	424±95	126±14
Whole blood (LSC)	45±5	BLQ	Blq
Plasma (LSC)	47±14	BLQ	Blq
LOQ	39	39	39
	Timepoint (% administered±SEM)		
	24h	96h	336h
Eye	0.056±0.027	0.020±0.001	0.009±0.002
Liver	0.232±0.034	0.046	NC
Uveal tract	0.035±0.007	0.019±0.001	0.009±0.002

Tissue distribution after a single (3 mg/kg) intravenous administration of (^{14}C-carbonyl) SR33589B to the male Sprague-Dawley rat. ***DIS0140***

Seven rats, 1 per sampling time, were given an intravenous injection of dronedarone containing 3.70 MBq/kg radioactivity. Sampling times were 1,4,8, 24, 96, 168 and 336 hours after dosing. The highest concentrations of radioactivity were seen in the lung. Half-life values were estimated for the liver, spleen and testes (69.8, 102.5 and 65.6 hours respectively).

The highest drug-associated radioactivity levels were observed in the lungs, hypophysis(data not shown), pineal body(data not shown), adrenal gland(data not shown), gastric wall, gut content and pancreas over the 1-4 hour sampling period. Radioactivity levels decreased from 8-24 hours. Radioactivity was found during that period in liver, spleen, kidney, Harder's gland, adrenal gland and testis.
At 168 hours, radioactivity was still observed in liver, spleen, adrenal, kidney and testis.
Residual radioactivity was reported to still be present in blood, liver, spleen and testis.
Although the sponsor specifically cited the hypophysis and pineal gland, I found only textual references to these sections.

This is more persistent radioactivity than was seen after oral administration to the female SD rat.

. Quantitative tissue distribution by Liquid Scintillation Counting : tissue concentrations expressed as mg Eq/kg of tissue

SAMPLING TIMES (hours)	1	4	8	24	96	168	336
Blood	0.195	0.108	0.036	0.032	0.078	0.080	0.078
Plasma	0.207	0.150	0.054	0.008	UDL	UDL	UDL
Brain	1.774	0.408	0.083	0.022	UDL	UDL	UDL
Kidney	3.774	1.896	0.738	0.146	0.053	0.026	UDL
Liver	4.454	2.461	1.524	0.453	0.076	0.037	0.007
Lung	12.273	6.282	1.074	0.063	0.009	UDL	UDL
Muscle	1.473	0.933	0.200	0.019	UDL	UDL	UDL
Myocardium	2.034	0.903	0.292	0.034	UDL	UDL	UDL
Spleen	4.718	2.717	0.831	0.110	0.044	0.024	0.012
Testis	0.214	0.249	0.172	0.118	0.038	0.015	0.004
Thymus	1.948	1.621	0.730	0.042	UDL	UDL	UDL
White fat	2.105	2.050	1.097	0.109	0.007	UDL	UDL

UDL = under detection limit

In Vitro Binding of [^{14}-carbonyl]- SR33589B to the Plasma Proteins of Rat, Mouse and Rabbit. ***LPR0873***

Plasma was obtained from male Sprague-Dawley rats, male CD-1 mice and female NZW rabbits. Pooled plasma was spiked with radiolabeled drug (specific activity of 3.47 MBq/mg (93.9 µCi/mg)) at concentrations of 50, 100, 500, 1000, 5000 and 10, 000 ng/ml. The mixtures were incubated at 37ºC for 2 hours at which time it was assumed that equilibrium was reached. Binding was determined by equilibrium dialysis.

Stability of the [^{14}C-carbonyl]- SR33589B in plasma was determined for each species after incubation at 37ºC for 2 hours by chromatographic analysis at concentrations of 100 and 1000 ng/ml during the equilibrium dialysis experiment.

Aliquots of plasma were extracted with methanol and dried under N_2. Reconstituted samples were subject to chromatographic analysis.

In all species, at all concentrations tested, [14C-carbonyl]-SR33589B was highly bound (>99%) to the plasma proteins. Over the tested range of 50-10,000 ng/ml, no concentration dependence was observed. Saturation of protein binding was also not observed.

In vitro binding of SR33589B to rat, dog, monkey maccaca and human plasma proteins. ***LPR0508***

Binding to rat, dog, monkey and human plasma proteins was studied by equilibrium dialysis carried out at 37ºC. Time to reach equilibrium and the diffusion of the compound through the dialysis membrane were studied in triplicate at 5 concentrations (0.001, 0.05, 0.1, 0.5 and 10 mg/l) and 5 times of incubation (0.5, 1,2,3 and 4 hours). Stability of SR33589B in human plasma was checked by HPLC analysis after incubation of samples for 2 and 4 hours at a concentration of 10 mg/l.

The drug was shown to be stable in the plasma under the conditions of the study for the timepoints and concentration examined. At all tested concentrations protein binding was >99.5% for rat, dog, macaque and human by equilibrium dialysis techniques. Using ultrafiltration, there was a suggestion of concentration dependence in the human plasma, the only data shown.

[SR33589B] mg/l	0.001	0.1	10
$F_{b,}$ human SD	87.52 (31.18)	99.15 (0.47)	99.14 (0.05)

In Vitro Binding of [^{14}C- Carbonyl]- SR35021A to the Plasma Proteins of Rat, Dog, Mouse, Rabbit and Human ***LPR0876***

Plasma was collected from male Sprague-Dawley rats, male Beagles, male CD-1 mice, female NZW rabbits, and male Caucasians. Radiolabeled drug was tested at concentrations of 50, 100, 500, 1000, 5000 and 10,000 ng/ml for 2 hour incubations at 37ºC.

Similar to the parent drug, under the conditions of the study the metabolite was highly protein bound in the species studied. At all concentrations tested, protein binding was reported as ≥98.7% across species.

BLOOD DISTRIBUTION AND PHARMACOKINETICS OF RADIOACTIVITY FOLLOWING SINGLE ORAL (30 MG/KG) OR INTRAVENOUS (10 MG/KG) ADMINISTRATION OF ^{14}C-LABELLED SR 33589B TO MALE AND FEMALE SPRAGUE DAWLEY RATS ***Study LPR0046***

Three animals/sex/sampling time/route were used. Oral doses were given in a suspension of 0.6% methyl cellulose. Intravenous doses were given as a solution in PEG400/sodium acetate buffer. Sampling times were:

IV: 0, 0.083, 0.5, 1,2,3,4,6,8,10,24,32, 48 and 72 hours

PO: 0, 0.5, 1, 1.5, 2,3,4,6,8,10, 24, 32, 48 and 72 hours.

Plasma was analyzed by direct liquid scintillation counting. Blood was analyzed by combustion prior to LSC.

Distribution of blood radioactivity results expressed as % in plasma : mean (SD)

Oral administration		
Time (h)	male	female
0.5	83(13)	(73(3)
1	71(11)	73(5)
1.5	72(7)	72(5)
4	69(5)	78(2)
10	77(17)	84(6)
Intravenous administration		
Time (h)	male	female
0.083	40(3)	60(25)
0.5	49(5)	57(7)
1	43(10)	63(20)
4	61(1)	78(24)
10	64(6)	71(2)

Mean pharmacokinetic parameters of plasma radioactivity

	oral		intravenous	
parameters	male	female	male	female
C_0 (mg Eq/l)			1.16	1.25
Cmax(mg Eq/l)	1.89(0.38)*	2.32(0.78)*		
Tmax (hour)	3	4		
$T_{1/2}$ (hour)	24.4	15.3	30.8	13.5

* standard deviation

The circulating half life was shorter in females than males following both routes of administration.

Blood distribution and pharmacokinetics of radioactivity following single oral (25 mg/kg) or single intravenous (2.5 mg/kg) administration of 14C-labeled SR33589B to male Beagle dogs. ***LPR0048***

Four males per route of administration were studied. The oral dose was administered as a suspension of 0.6% methyl cellulose with. The intravenous material was prepared in PEG400/sodium acetate. The radioactivity administered by either route was 1.85 x 10^3 KBq/kg. Sampling times were:

Oral: 0, 0.5,1,1.5, 2,3,4,6,8,10, 24, 32, 48, 54, 72, 96, 120 and 168 hours.
IV: 0, 0.083, 0.25, 0.5, 1, 2,3,4,6,8, 10, 24, 32, 48, 120 and 168 hours.

Plasma was analyzed by direct LSC. Blood was subject to combustion prior to LSC.

Percentage of radioactivity in plasma: Mean (SD)

Time (hours)	oral	Intravenous
0.083		52.6(6.2)
0.5	67.9(8.8)	53.3(6.7)
1	74.9(5.1)	57.1(3.3)
4	62.7(3.1)	51.9(10.8)
10	58.6(13.0)	49.7(2.3)
24	62.3(29.9)	49.9(18.5)
48	52.9(39.1)	27.5(11.7)

Pharmacokinetic parameters of plasma radioactivity: mean (SD)

	oral	intravenous
Cmax(mg Eq/l)	2.63 (1.27)	
Co (mg Eq/l)		0.62(0.11)
Tmax (hour)	2.38(1.11)	
$T_{1/2}$ (hour)	11.45(5.26)	35.33(7.02)

The half- life was longer with intravenous administration. In the previous report using rats, intravenous administration also had a half life as long or longer than that found using oral administration. The sponsor presumed the shorter t1/2 after oral vs IV administration to be due to lower levels of absorption and therefore a more rapid decrease to circulating levels below the limits of detection.

Blood distribution and pharmacokinetics of radioactivity following single intravenous (1mg/kg) administration of 14C-labelled SR33589B to male Macaca monkeys. ***LPR0047***

Four male Macaca fascicularis were used. SR33589B in aqueous solution of mannitol and monobasic sodium phosphate was used to deliver a dose of 1 mg/kg with radioactivity of $1.85x10^3$. Blood and plasma were collected at 0, 0.083, 0.25, 0.5, 1,2,3,4,6,8,10,24, 48, 120 and 168 hours. Analysis was by combustion and LSC.

Distribution of blood radioactivity
(% in plasma ±SD)

Time (hour)	%plasma
0.083	51.54±6.40
0.5	47.39±6.60
1	44.42±6.99
4	44.23±7.21
10	50.34±12.12
24	64.51±9.56

PK parameters of plasma radioactivity

	Mean(SD)
C0 (mg Eq/l)	0.328(0.071)
T1/2(hour)	66.9(6.0)

A long circulating half-life is seen in the macaque following intravenous administration.

Fetal tissue distribution of radioactivity following a single (100mg/kg) oral administration of (Carbonyl-14C) SR33589B to the pregnant Sprague-Dawley rat. ***DPK0054***

Two pregnant females were used per sampling time. Blood, plasma and tissues were collected at 6 and 24 hours after dosing on GD18. The animals received a single oral dose of 90 mg/kg (non-salified compound) in 0.6% methylcellulose with 3.9±0.084 MBq/kg.

As shown in the sponsor's table below, radioactivity was well represented in the female reproductive tract, placenta and fetal liver. At 24 hours after dosing there was a greater concentration of radiolabel in the mammary gland than in the myocardium. The radioactivity levels in the uterus had not declined from 6 hours. It appears that there may be retention of drug or accumulation in the uterus and possibly other parts of the reproductive system.

Distribution pattern

Sampling times (h)	Tissues
6	• In pregnant females : Adrenal gland > Spleen > Liver > Lung > Kidney > Mammary glands > Harder's gland > Myocardium > Hypophysis > Ovary. Blood and brain radioactivity close to the background noise of the detector. • In fetal area : Trophoblast > Placenta > Uterus (uterine wall and/or decidua) > Fetus > Fetal liver > Amniotic fluid
24	• In pregnant females : Harder's gland > Lung > Thymus > Mammary glands > Liver > Myocardium. Blood and brain radioactivity close to the background noise of the detector. • In fetal area : Trophoblast > Uterus (uterine wall and/or decidua) > Placenta. Fetus, fetal liver and amniotic fluid radioactivity levels close to the background noise of the detector.

. Individual and mean tissue concentrations on day 18 of gestation (results expressed as mg Eq. non salified compound/kg).

Sampling times (h)	6			24		
Animal number	CA28	CA39	Mean value	CA32	CA40	Mean value
Blood	2.868(*)	3.923(*)	3.396(*)	1.339(*)	UDL	1.339(*)
Liver	64.046	89.05	76.552	7.801	16.898	12.350
Mammary gland	19.249	34.825	27.037	5.504	15.782	10.643
Ovaries	14.405	26.516	20.461	2.132(*)	4.883	3.508(*)
Plasma [1]	2.107	3.055	2.581	0.281	0.697	0.489
Uterus [2]	12.628	34.552	23.590	28.999	23.673	26.336
Trophoblast	20.673	39.093	29.883	10.881	15.624	13.253
Placenta	11.038	17.847	14.443	1.528(*)	5.837	3.683(*)
Amniotic fluid	UDL	UDL	NC	UDL	UDL	NC
Fetus	UDL	2.659(*)	2.659(*)	UDL	UDL	NC
Fetal liver	2.355(*)	3.703(*)	3.029(*)	ND	ND	ND

UDL = Under detection limit
(*) = Under quantification limit
[1] = Values issued form LSC
[2] = Uterine wall and/or decidua
NC = Not calculated
ND = Not determined
LOD = 1.205 mg Eq./kg
LOQ = 4.018 mg Eq./kg

Placental transfer of radioactivity after a single oral (60 mg/kg expressed as unsalified compound) administration of [^{14}C-Carbonyl]-SR33589B to the pregnant New Zealand rabbit (Quantitative Whole Body Autoradioagraphy) ***PLT0037***

One pregnant NZW rabbit was used per timepoint. After receiving a single oral dose of radio-labeled material, sagittal whole body sections were taken through each animal at 4, 24 and 48 hours. The sections were analyzed by quantitative whole body autoradiography.

Maternal radioactivity at several timepoints

Maternal tissue Sample	Timepoint		
	4 h	24 h	48 h
Amniotic fluid	Blq	Blq	Nd
Blood	1383	Blq	Blq
Deciduas	944	764	Nd
Liver	36853	3686	1203
Ovaries	1024	950	599
placenta	2179	861	Nd
Trophoblast	2800	908	Nd
Uterus	1086	472	Nd
Uterine luminal fluid	blq	blq	nd

Fetal radioactivity at several timepoints

Fetal tissue Sample	Timepoint		
	4 h	24 h	48 h
Whole fetus	Blq	Blq	Blq
Blood	Blq	Blq	Nd
Brain	Blq	Blq	Nd
Eye	Blq	Blq	Nd
Gastric content	Blq	744	636
Gut content	762	16338	55588
Kidney	Blq	Blq	Nd
Liver	Blq	Blq	Nd
lung	Blq	Blq	Nd
Muscle	Blq	Blq	Nd
Myocardium	Blq	Blq	Nd
tissue	Blq	Na	Blq
loq	375	367	470
Whole blood (LSC)	1454	117	Blq
Plasma (LSC)	1974	299	Blq
loq	75	75	75

Lsc= liquid scintillation counting

This small sample size study provided evidence of fetal exposure to maternally administered drug in that the concentration of radioactivity in the fetal gastric contents increased over the course of the study. Is this present due to movement from fetal blood through a liver capable of making bile?

Toxicokinetic study, complementary to the study for effects on embryo-fetal development in the rabbit. ***DIV0914***

Three mated female New Zealand White rabbits per group were given oral doses of 0, 20, 60 or 200 mg/kg/day of dronedarone in 0.6% methylcellulose from GD6 to GD18. Blood samples were drawn the first and last day of dosing at 0, 1,2,4,8 and 24 hours after dosing. At time or euthanasia, 4 females were not pregnant: 1 LD f, 1 MD f and 2 HD f. We do not know if these were early or late losses or other.

Summary of TK parameters Days 6 and 18 of gestation

day	Dose Mg/kg/day		Tmax hour	Cmax Ng/ml	AUC_{0-24} Ng.h/ml
GD 6	20	Mean ±SD	1	14.3±8.1	87.5±11.4
		CV%		57	13
GD 6	60	Mean ±SD	1	60.7 (n=1)	461.8
		CV%		NA	
GD 6	200	Mean ±SD	4	323.0±170.4	4497.2±2469.7
		CV%		53	55
GD 18	20	Mean ±SD	1	5.2±1.1	60.6±9.3
		CV%		21	15
GD 18	60	Mean ±SD	4	46.1	532.2
		CV%		NA	
GD 18	200	Mean ±SD	8	775.3±85.6	13852.7±3827.8
		CV%		11	28

Based on this small sample size it can be said that oral dosing produced systemic exposure in the pregnant rabbits used for this study.

2.6.4.5 Metabolism

Interspecies variability in SR33589B metabolism by hepatic microsomal fractions ***MIV0143***

Hepatic microsomal fractions were obtained from Swiss CD1 mice, Sprague-Dawley rats, Hartley guinea pigs, New Zealand rabbits, Beagle dogs, Yucatan minpigs, macacca and baboon primates and humans. After a 60 minute incubation ± NADPH, the samples were analyzed for parent drug and some metabolites. In the absence of NADPH SR33589B did not disappear and metabolites did not appear. With NADPH, numerous metabolites were reported. These were numbered based on elution order.

The percentages of biotransformation under the conditions of the study were

species	Primary metabolites	% of biotransformation
Swiss CD1 mouse	6,8	35.7
SD rat	1,6	39.8±5.6
Hartley guinea pig	6,8	43.3
NZW rabbit	6,8	40.9±1.9
Beagle	1,6	54.5±7.5
Yucatan minipig	1,8	50.3±12.0
Macacca monkey	1,8	79.5±2.1
Baboon	1,8	78.8±2.0
Caucasian human	1,8	41.8±14.9

Metabolite 8 = SR35021

Profile and identification of plasma, urine and fecal metabolites following a single and repeated oral administration (75 mg/kg/day) of [14C]-SR33589B to male Swiss CD1 mice. ***MET 0462***

Drug was administered as a suspension in 0.6% methyl cellulose. Mice received either a single dose or repeated daily dosing over 14 days.
Blood/plasma samples collected: pre tx days 1,7,10 and 14
Days 1 and 14: 0.5, 2,4,8 and 24 hours after dosing
Urine, feces and cage washes: 24 hour fraction after dosing days 1,7,10 and 14
Samples were analyzed by HPLC-MS with radioactivity detection and HPLC/MS/MS for identification.

Excretion data for total radioactivity

sample	period	Amount as mg-Eq	% of daily excreted radioactivity
urine	Day 1: 0-24	0.70	3.32
	Day 7: 0-24	0.96	3.56
	Day 10: 0-24	1.07	4.50
	Day 14: 0-24	1.09	4.68
feces	Day 1: 0-24	20.4	96.7
	Day 7: 0-24	26.0	96.4
	Day 10: 0-24	22.7	95.5
	Day 14: 0-24	22.2	95.3

PK parameters for total radioactivity, SR33589 and SR35021 in plasma

sample	day	Tmax hours	T1/2 hours	Cmax	AUCτ	AUC	R_{ac}*	R_{AUC} SR/TR**
radioactivity	1	2	2.9	5850		33500		
	14	2	4.4	5890	43400		nc	
SR33589B	1	0.5	2.1	1400	8390	8390		0.25[a]
	14	0.5	2.4	1930	8120		0.97	0.19[b]
SR35021	1	2	2.2	991	6150	6150		0.18[a]
	14	2	2.3	1010	7580		1.23	0.17[b]

*accumulation ratio between day 14 and day 1; **ratio SR33589/total radioactivity
[a]calculated from AUC on Day 1; [b]calculated from AUCτ on day 14

Under the conditions of the study, there was no detected change in pharmacokinetic parameters from day 1 to day 14. Greater than 90% of the radioactivity was recovered primarily in the feces regardless of duration of dosing. There was no qualitative change in the metabolite profile over the course of the study either.

Plasma, urinary, tissue and fecal metabolites following single and repeated oral administrations (10 mg/kg/day) of [^{14}C]SR33589B to male Sprague-Dawley rats. ***RME0003***

Rats received either a single oral dose or repeated dosing for 14 days.
Samples collected:
Plasma: day 1 pre-tx, 0.5, 1,2,4,8,12,18,24,48,72,120 and 168 hours after treatment
Days 7 and 10: pre-tx
Day 14: pre-tx, 0.5, 1,2,4,8,12,18, 24, 48, 72, 120 and 168 hours after tx
Urine, feces: 24 hour fractions over 7 days after the day 1 dosing
24 hour fractions after dosing on day1,6, and 9 and daily over 7 days after dosing on day 14.
Tissues: lung, myocardium and liver collected 4 hours and 24 hours after dosing on day 24

The sponsor stated that due to low specific radioactivity, no interpretable radiochromatograms for plasma could be obtained. Qualitative metabolic profiles were based on a reconstructed ion chromatogram during MS analysis. Qualitative differences from day 1 to day 14 were not detected under these conditions. HPLC radiochromatograms for urine showed similar metabolic profiles for both days of analysis. The hydroxyl-SR35021 metabolite was the main excreted compound in urine, increasing from 22% on day 1 to 35% day 14 of all radioactive peaks detected. Unchanged parent drug was not reported as a significant presence in the urine. However, in the feces, unchanged drug accounted for ~5%(day6) and ~12%(day 1) of all radioactive peaks. Peaks 8 and 9, mono-hydroxy SR35021 derivatives, were the main excreted species in the feces. Peak 8 accounted for 11-17% of total excreted compound and peak 9 accounted for 20-27% of all detected peaks.

tissue	Primary species	amount
Liver	Peak 9 :mono-hydroxy SR35021	24% of all radioactive peaks
Lung	UD:SR33589B Peak 21: SR35021	Relative percentages not given
myocardium	UD:SR33589B Peak 21: SR35021	Relative percentages not given

The main routes of biotransformation were thus reported as:
Hydroxylation and oxidation of parent compound
N-debutylation leading to SR35021 and subsequent hydroxylation/oxidation
Oxidative deamination leading to SR90154 and subsequent hydroxylation
Cleavage between benzofuran ring and carbonyl group yielding benzoic acid derivatives

The plasma radiochromatograms were reported as of poor quality but that a conclusion had been reached that there were no qualitative changes between day 1 and day 14. The two main metabolites were postulated as a benzoic acid and propionic acid (SR90154).

No qualitative or quantitative differences were reported for day 1 to day 14. Parent drug was not detected in the urine samples. The main metabolite reported was OH-SR35021. The main excreted compounds identified in the feces were SR33589B, SR35021 and 2 hydroxylated SR35021 isomers.

Both SR33589B and SR35021 were found (as well as other metabolites) in the lung, liver and myocardium.

Profile and identification of plasma metabolites following single oral (30 mg/kg) or single intravenous (10 mg/kg) administration of ^{14}C-labeled SR33589B to male and female Sprague-Dawley rats ***Met0138***

Three rats/sex/route/sampling time were given single oral or intravenous doses of ^{14}C-SR33589B. Oral doses were suspended in 0.6% methyl cellulose. Intravenous doses were dissolved in PEG400/sodium acetate 0.01M, pH 4.9. Plasma was sampled at the following times:

Oral: 0, 0.5, 1,1.5, 2,3,4,6,8,10, 24,32,48 and 72 hours.
IV: 0, 0.083, 0.5, 2,3,4,6,8,10,24, 32,48 and 72 hours.

Samples were analyzed by HPLC with UV, radioactive and MS detection. Plasma samples after 10 hours were not analyzed due to low radioactivity concentrations.

In both sexes, parent drug was detected up to the 10-hour sampling time. After oral treatment, metabolite 1, a carboxylic acid derivative due to N-dealkylation, not the parent drug, was the predominant circulating species in either sex. In males, this metabolite comprised 70% (relative percentage but relative to what is not clear) at 0.5 hours down to 16% at 10 hours. In females this metabolite comprised 42% (relative percentage) at 0.5 hours and 84% at 10 hours.

After intravenous administration, SR33589B was the main compound found in males at all times and in females up to 2 hours after dosing. The relative percentage in males ranged from 88% at 0.083 hours to 43% at 4 hours and back up to 60% at 10 hours. In female the relative percentage of SR33589B ranged from 86% at 0.083hours down to 30% at 10 hours.

Profile and identification of urinary metabolites following a single oral (30 mg/kg) or intravenous (10 mg/kg) administration of ^{14}C-labelled SR33589B to male and female Sprague-Dawley rats. ***Met0139***

This is the analysis of urinary metabolism from the preceding report. Sampling times for urine were 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours. Analysis was by HPLC with UV, radioactive and MS detection. For each administration route and each sex, the urinary metabolite profile was performed at random on one animal over a 0-48 hour period of collection.

After oral administration the parent drug was not detected in either sex except in the 24-48 hour fraction. The sponsor did not offer an explanation for this. The possibility of column contamination is one consideration. Parent drug was extensively metabolized in both sexes although more radioactive peaks were reported for the females than the males. The same major compound was reported for both sexes. That was the mono-N-debutyl-hydroxy (on the benzofuran ring). Enzymatic hydrolysis produced little change, suggesting that most radioactive

peaks were excreted in the free form. No major qualitative differences in the urinary profile were reported regardless of sex or route of drug administration.

Profile and identification of biliary metabolites following single oral (30 mg/kg) or intravenous (10 mg/kg) administration of 14C labeled SR33589B to male and female Sprague-Dawley rats. ***MET0140***

Bile duct cannulated rats, 5/sex/route were used. Samples were collected 0-1, 1-3, 3-6, 6-24 and 24-48 hours. Analsysis was by HPLC with UV, radioactive and MS detection.

Biliary excretion as a percent of the administered dose was greater in both sexes following intravenous dosing. Parent drug was not detected in either sex or after either administration route. Part of the sponsor's summary is reproduced below.

Period (hours)	oral		intravenous	
	male	Female	male	Female
0-1	0.25 ±0.15	0.30±0.07	5.41±1.44	3.93±2.06
0-24	36.68±9.16	41.82±3.92	69.25±3.15	60.79±8.66
0-48	43.24±11.12	45.67±4.05	71.97±3.59	66.01±8.02

Some of the biotransformation identified included taurine, glucuronide and sulfo-conjugates, butyric acid derivatives, O-propanoic acid derivatives, various hydroxylations.

Profile and identification of plasma, urine and fecal metabolites following a single oral administration (60 mg/kg) of [14C]-SR33589B to the female New Zealand white rabbit. ***MET0533***

Two groups of 4 females/group. Plasma was collected pre-tx,1,2,4,8,12,24,48 and 72 hours after administration. Urine, feces and cage washes: 24 hour fractions before dosing and daily for 72 hours. Analysis was HPLC with UV, radioactivity and MS detection.

The majority of drug-associated radioactivity was excreted in the feces: 69% of the given dose in the first 24 hours and a cumulative total of 88% by 72 hours. Nine percent of the given dose was recovered in the urine by 24 hours. This changed only minimally by 72 hours.
The pharmacokinetic parameters calculated are summarized below:

parameter	blood	Plasma
Cmax (µg-Eq/g)	1.53±0.413	0.874±0.178
Tmax (hours)*	3[1-4]	2.5[1-4]
$T_{1/2}$ (hours)	4.35±0.45	4.18±0.497
T_{last} (hours)*	12[4-24]	12[8-24]
AUC_{last} (µg-Eq.h/g)	7.40±1.10	6.04±1.33
AUC (µg-Eq.h/g)	NC	NC

*median[range]

Summary of main metabolites

matrix	Metabolites
plasma	SR33589B 4%, keto-and hydroxyl SR33589B 21%, keto and hydroxyl-N-debutyl SR33589B 19%, OH-propionic acid derivative SR90154 17%
urine	Main metabolites corresponded to the main metabolites in plasma. Parent drug not identified
feces	Keto and hydroxyl-N-debutyl SR33589B 5%, keto and hydroxyl SR33589B 24%, SR33589B 12%

Profile and identification of plasma, urinary and fecal metabolites following single and repeated oral administrations (15 mg/kg/day) of [14C-carbonyl]-SR33589B to male dogs. ***RME0009***

Dogs received a single dose and then daily oral dosing for 14 days with a 35 day washout period.
Blood samples were collected before treatment days 1,7,10 and 14
Day 1: 0.5, 1,2,4,8,24,48,72,96,120,144 and 168 hours post-dosing
Day 14: 0.5, 1,2,4,8,24,48,72,96, 120, 144 and 168 hours after dosing.
Urine, feces and cage washes: 24 hour fraction after dosing on days 1,7, 10,and 14 by 24-hour fractions over 168 hours after dosing days 1 and 14.

Qualitative differences were not reported between days 1 and 14.

Summary of main metabolites per matrix

matrix	metabolite	% of total detected compounds in matrix	
		Day 1	Day 14
plasma	SR90154	31%	41%
	SR33589	19%	20%
\			
urine	Dihydroxy-SR33589	20%	13%
	Monohydroxy-SR35021	20%	17%
	Parent compound not seen		
feces	SR33589	7%	6%
	Dihydroxy-SR35021	21%	16%
	Monohydroxy-SR35021	20%	22%

Profile and identification of plasma metabolites following a single oral (25 mg/kg) or a single intravenous (2.5 mg/kg) administration of (14C)-SR 33589B to male dogs. ***MET0237***

Sampling times after oral dosing were 0, 0.5, 1,1.5, 2,3, 4,6,8, 10, 24,32, 48, 54, 72, 96, 120, 168 hours. Sampling after intravenous dosing: 0, 0.083, 0.25, 0.5, 1,2,3,4,6,8,24,32,48, 120 and 168 hours.

After single oral administration to male dogs the main circulating metabolite was O-propanoic SR33589 accounting for 23% at 10 hours to 74% (1 hour) of all detected metabolites. SR33589 and SR35021 were detected as minor metabolites.

After single intravenous administration to male dogs, unchanged drug was detected as the main circulating compound over the first 10 hours accounting for 19%- 76% of all detected metabolites.

Profile and identification of urinary metabolites following single oral (25 mg/kg) and intravenous (2.5 mg/kg) administrations of (14C)-SR33589B to male dogs. ***MET0238***

Sampling times for urine were 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours. Aliquots of urine were treated with β-glucuronidase to assess conjugation.

The same qualitative profiles were obtained with both routes of administration but with quantitative differences. At the time of the report, the structures of the urinary metabolites had not been confirmed.

Profile and identification of plasma metabolites following single intravenous (1 or 8 mg/kg) administration of 14C-labelled SR33589B to male macaca monkeys ***MET0141***

Four males received drug in mannitol/monobasic sodium phosphate solution (group 1) and 2 males received drug in PEG/sodium acetate solution (group 2). Blood (plasma) samples were collected for group1 at 0, 0.083, 0.25, 0.5, 1,2,3,4,6,8,10,24, 48,120 and 168 hours. Samples were collected from group 2 at 0, 2,6 and 24 hours.

One main radioactive peak was detected with HPLC retention time corresponding to that of SR33589. Other minor peaks were detected that included SR35021 and a carboxylic acid formed by deamination and oxidation of the parent compound.

Profile and identification of urinary metabolites following single intravenous (2.5 or 8 mg/kg) administration of 14C-labelled SR33589B to male macaca monkeys. ***MET0142***

Four males were in group 1 and 2 males in group 2. Both groups received drug formulated in PEG/sodium acetate. Sampling times for group 1 were 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168hours. Group 2 was sampled from 0-24 hours.

Parent compound was not detected in the urinary samples at any time point or at either dose. Enzymatic analysis showed that the metabolites were not conjugated. The main peaks were mono- or di- hydroxylated on the benzofuran ± mono or di-N-debutylation. Final characterization of the metabolites had not been completed at the time of writing of the report.

Effects of SR33589B repeated oral administration (375,450 mg/kg/day) during 14 days on various liver monooxygenase activities in CD-1(ICR)BR mouse. ***TIN0089***

Animals from the tk satellite group, 10/sex/group and 3 pools of 2 animals/sex/group corresponding to 0,1, and 2 hours post-dose for the enzyme evaluation (6 animals per group) were used. They were treated daily for 14 days. Liver samples were collected at time of necropsy and microsomes prepared.

Activity CYP450 involved (nanomoles product/min/mg of protein)	Effect: in males		
	0	375	450
7-ethoxyresorufin O-dethylase CYP1A			
7-pentoxyresorufin O-dealkylase CYP2B	0.074	0.110	0.166*
Aminopyrine N-demethylase CYP2C-3A			
Aniline hydroxylase CYP2E			
Erythromycin N-demethylase CYP3A	4.29	8.20*	8.64*

*p<0.05

Activity CYP450 involved (nanomoles product/min/mg of protein)	Effect: in females		
	0	375	450
7-ethoxyresorufin O-dethylase CYP1A			
7-pentoxyresorufin O-dealkylase CYP2B			
Aminopyrine N-demethylase CYP2C-3A	9.92	11.81	12.40
Aniline hydroxylase CYP2E			
Erythromycin N-demethylase CYP3A	3.51	5.36*	6.70*

*p<0.05

A significant dose-related increase in erythromycin N-demethylase was observed in both sexes (CYP3A).

Effects of a 2-week repeated oral administration of SR33589B (30, 70 and 160 mg/kg/d) on various liver enzyme activities in Sprague-Dawley rats. ***TIN0045***

Ten animals/sex/group and 5 animals/sex for enzyme evaluation. Animals were treated daily for 2 weeks with drug administered as a suspension in 0.6% methylcellulose.

Activity CYP450 involved (nanomoles product/min/mg of protein)	Effect in males			
	0	30	70	160
7-ethoxyresorufin O-dethylase CYP1A				
7-pentoxyresorufin O-dealkylase CYP2B				
Aminopyrine N-demethylase CYP2C-3A	3.86	3.81	4.19	2.47*
Aniline hydroxylase CYP2E	0.71	0.80	0.93*	0.68
Erythromycin N-demethylase CYP3A				

*p<0.05

Activity CYP450 involved (nanomoles product/min/mg of protein)	Effect in females			
	0	30	70	160
7-ethoxyresorufin O-dethylase CYP1A				
7-pentoxyresorufin O-dealkylase CYP2B				
Aminopyrine N-demethylase CYP2C-3A	2.88	2.04*	2.11*	2.64
Aniline hydroxylase CYP2E				
Erythromycin N-demethylase CYP3A	1.42	1.26	1.18	1.85*

*p<0.05

No consistent dose-related effects were seen. Erythromycin N-demethylase showed a slight and statistically significant increase in the HD females. Amino-N-demethylase activity showed a statistically significant decrease at the LD and MD and a non-significant decrease at the HD. The inconsistency and small magnitude of effects make it unlikely that they are of biological significance.

Effect of a 2-week repeated oral administration of SR33589B (25, 60 and 140 mg/kg/d) on various liver enzyme activities in Beagle dogs. ***TIN0046***

Three animals /sex/group received drug daily for 14-15 days.

There were no discernible effects on the liver/bodyweight ratio or CYP450 content expressed as nanomoles/mg protein. There were minimal and non-significant effects upon enzyme activity.

Under the conditions of the study, 2 weeks of exposure to the drug caused no detectable changes in the CYP450 enzymes examined.

2.6.4.6 Excretion

Pharmacokinetics and excretion balance after single and repeated oral (10 mg/kg/day) administration of [14C-carbonyl]-SR33589B to male Sprague-Dawley rats. ***RDS0008***
Animals used:
5 males for excretion after single oral dose
5 males for excretion following multiple oral doses
33 males for plasma kinetics after single oral dose
42 males for plasma kinetics after multiple oral doses

Whole blood and plasma samples were obtained :

Day1: 0.5, 1,2,4,8, 12,24,48,72,120 and 168 hours
Day 6: 24 hours after dosing
Day 9: 24 hours after dosing
Day 13: 24 hours after dosing
Day 14: 0.5, 1,2,4,8,12,18, 24,48, 72, 120 and 168 hours after dosing.

Urine, feces and cage wash samples:
After single dosing: 0-24 hours and 24 hour fractions up to 168 hours after dosing
After single and repeated dosing: 24 hours post-dose 1, 24 hours post dose 6, 24 hours post dose 9, 24 hours post-dose 13 and 24 hour fractions until 168 h post-dose 14.

Expired air: 0-24 and 24-48 hours post single dose.

Summary of PK parameters for male rats

compound	Tmax (hour)	Cmax (ng/ml) or ng Eq/ml	AUC_{last} ng h/ml	T1/2 h
Radioactivity day 1	4	204	1606	3.0
Radioactivity day 14	2	398	2421	2.7
SR33589 day 1	1	17.5	110	2.4
SR33589 day 14	1	38.0	252	2.6
SR35021 day1	8	2.8	24	Nc
SR35021 day14	4	9.8	70	2.4

After a single dose, 76% of the radioactivity was excreted in the feces after 24 hours. Cumulatively 93% of the radioactivity was excreted in the feces after 168 hours. 2.4% was excreted in the urine after 24 hours. This did not change cumulatively over 168 hours.
The same findings occurred after repeat dosing. Cumulatively, 114% of the administered radioactivity was excreted by 168 hours after the last dose while 3.3% was excreted in the urine over the same time frame. Terminal half-lives were similar between radioactivity, parent drug and metabolite. There was a tendency to higher AUC values over the 14 day study period.

In the text of the report it was noted that mean concentration of total radioactivity in the heart, liver and lungs was ~13, 5 and 20 times higher respectively at 4 hours post dose day 14 than at 24 hours post dose day 1. Tissue accumulation was thus indicated although definitive studies to confirm and quantify this have not been performed.

Excretion balance following a single oral (30 mg/kg) or a single intravenous (10 mg/kg) administration of 14C-labelled SR33589B to male and female Sprague-Dawley rats. ***EBA0056***

After a single dose of drug, samples were collected as follows:

Urine: 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours
Feces: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours
Carcass: 168 hours

Oral administration: Results expressed as % of the dose given

	urine	feces	carcass	Total
Male 0-24 hrs	4.94±0.88	66.85±2.32		
Male 0-168	5.61±1.11	71.81±2.71	12.87±1.38	90.30±3.47
Female 0-24hr	4.60±0.60	65.92±5.73		
Female 0-168hr	5.25±0.72	72.33±6.60	15.72±2.29	93.29±5.43

Intravenous administration: Results expressed as % of the dose given

	urine	feces	carcass	Total
Male 0-24 hrs	5.05±0.54	70.94±7.98		
Male 0-168	6.18±0.62	78.75±9.40	5.00±2.18	89.93±11.07
Female 0-24hr	5.05±1.18	74.42±5.74		
Female 0-168hr	6.52±1.18	86.83±4.91	5.26±2.23	98.61±6.59

Consistent with other studies, radioactivity was primarily excreted via the feces.

Enterohepatic recirculation of radioactivity following a single intravenous (10 mg/kg) administration of [^{14}C]-SR33589B to male Sprague-Dawley rats. ***MET0464***

Route of administration was either a single intravenous dose to bile-duct cannulated animals (grp 1) or single intraduodenal administration to bile-duct cannulated animals of pooled bile from grp 1. Samples collected were urine, bile, feces and cage washes over 24 hours after dosing. These were analyzed for total radioactivity.

Mean±SEM excretion for total radioactivity over 0-24 hours after dosing in male Sprague-Dawley rats

sample	Intravenous (n=4)	Intraduodenal (n=5)
Urine	6.77±1.57	2.09±0.08
Feces	7.00±0.84	67.1±3.60
Bile	64.1±6.55	9.16±0.32
Cage washes	0.34±0.10	0.01±0.01
total	78.2±6.74	78.4±3.59

After intravenous administration, the main route of elimination was the bile, accounting for about 64% of the administered radioactivity. SR33589 was reported to be detectable at less than 1%. Most recovered compounds were reported to be free forms. Two radioactive peaks were excreted as conjugated derivatives: a taurine conjugate and a glucuronide peak. The sum of these was approximately 9% of all radioactive peaks detected. The main radioactive peaks were reported to correspond to mono- and dihydroxylated derivatives of SR35021.

When the bile from the intravenously-dosed animals was collected and injected intraduodenally into another group of rats, the main route of elimination was again the feces. The main peaks recovered included a taurine conjugate (~20% of detected peaks), an hydroxylated SR35021 (~13%) and an hydroxyl-propionic acid derivative (~12% of detected peaks).

Pharmacokinetics and excretion balance after single and repeated oral (15 mg/kg/day) administration of[14C-Carbonyl]SR33589B to male dogs. ***RDS0009***

A single day of treatment was followed by 14 days of daily treatments. Urine and feces were collected predose and at 24 hour intervals up to 168 hours post-dose. Whole blood and plasma were collected pre-dose and at 0.5, 1,2,4,8, 24,48, 72, 96, 120, 144 and 168 hours post-dose.

For the repeated dosing protocol, urine, feces and cage wash were collected for the periods 0-24 hours on dosing days 7 and 10 and for 0-168 hour post-dose after day 14 at 24 hour intervals. Whole blood and plasma were collected pre-dose days 7, 10 and 14 and at intervals following dosing on day 14.

Mean±SEM PK parameters for total radioactivity

day	Tmax (h)	Cmax (ngEq.h/ml)	T1/2 (h)	AUC0-τ (ngEq.h/ml)
1	2(1-2)	2090±114	5.1±0.3	9933±329
14	2(1-2)	1450±126	14.6±1.1	10194±683
Mean±SEM PK parameters for SR33589				
1	1.5[1-2]	199.3±25.2	4.0±0.1	893±157
14	2[1-2]	246.9±53.7	4.4±0.4	1479±359
Mean ±SEM PK parameters for SR35021				
1	2[1-2]	22.4±4.2	1.7±0.1	NC
14	2[1-2]	29.0±6.2	2.2±0.1	148±38

Single dose: Mean±SEM excretion of total radioactivity as % of administered dose

Day 1		urine	feces	Cage wash	Total
	0-24 h	3.34±0.15	73.86±2.89	1.49±0.04	78.69±3.02
	0-168 h	3.74±0.12	90.83±1.01	2.12±0.06	96.68±0.88
Repeat dose: mean±SEM excretion of total radioactivity as % of daily administered dose					
Day 7	0-24h	3.36±0.28	80.21±6.33	0.45±0.03	84.02±6.33
Day 10	0-24h	3.21±0.33	76.44±11.07	0.62±0.15	80.28±28
Day 14	0-24h	3.00±0.24	76.68±2.92	0.65±0.04	80.33±2.69

Total radioactivity was greater than SR33589 concentration. Unchanged drug seemed to account for 9-14% of total circulating radioactivity. From day 1 to day 14 there was an increase in AUC_{0-t} of 1.6x with minimal increase in Cmax. SR35021 accounted for approximately 1/10 the value of SR33589 (0.8- 1.4% of total radioactivity). Following either single or repeat dosing, feces was the main route of excretion with urine accounting for only 3-4% elimination.

*Excretion balance following single oral (25 mg/kg) or single intravenous (2.5 mg/kg) administration of 14C-labelled SR33589B to male Beagle dogs.**EBA0092***

Urine and feces were collected as follows:
Urine: 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours
Feces: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours

Oral administration: values as % of the dose given ±SD

	0-24hours	0-48 hours	0-168 hours
Urine	3.32±1.93	5.53±2.49	6.35±3.18
Feces	32.73±32.79	58.83±35.28	78.33±10.39
total	36.05±33.84	64.36±33.33	84.68±10.63
Intravenous administration			
Urine	4.20±1.59	5.00±1.41	5.42±1.46
Feces	26.56±19.30	55.46±4.07	64.75±5.83
total	30.77±20.27	60.46±4.26	70.17±5.54

Consistent with other studies, excretion, even after IV administration, was mainly through the feces. By 168 hours approximately15- 30% of the radiolabel had not yet been excreted.

Excretion balance following a single intravenous (2.5 mg/kg) administration of 14C-labelled SR33589B to male macaca monkeys. ***EBA0057***

Four male Cynomologous monkeys received IV boluses of radio-labeled drug. Urine and feces were collected as follows:

Urine: 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours
Feces: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours

Intravenous administration: values expressed as % of the given dose ±SD

	urine	feces	Total
0-24h	2.9±1.6	9.1±10.1	12.1±8.9
0-72h	3.8±1.9	77.8±6.9	81.6±5.8
0-168h	4.2±2.1	84.6±5.0	88.8±4.9

Consistent with other studies, the main route of excretion was via the feces. Compared to other species, there was less excretion within the first 24 hours in this small sample-size study. Again consistent with other studies in other species, excretion was not complete at 168 hours after dosing.

The secretion of total radioactivity in milk of lactating albino rats following a single oral (30 mg/kg) administration of [^{14}C-carbonyl]-SR33589B. ***MIL0003***

The dams received a single oral dose on day 11 after parturition. Plasma, milk and pups gastric content was sampled 2, 4 and 24 hours post dose.

The secretion of total radioactivity in milk of lactating albino rats following a single oral (30 mg/kg) administration of [^{14}C-carbonyl]-SR33589B

	Timepoint (ngEq unsalified compound/g SEM))		
	2h	4h	24h
Milk	3814	5614	110±17
Plasma	1539±299	1362±154	Blq
Pup gastric contents	Blq	873±63	3531±226
Milk:plasma ratio	2.3	4.6	3.3

At all points of determination there was a greater concentration of drug in milk compared to plasma. Total radioactivity was also found in the stomachs of pups. From the information in this study it can be said that the drug and/or its metabolites find their way into the milk. It can't be determined from available material if there is an active secretion or sequestration process.

2.6.4.7 Pharmacokinetic drug interactions : None submitted

2.6.4.9 Other Pharmacokinetic Studies: no other studies submitted

2.6.4.10 **Discussion and Conclusions** The absorption, distribution, metabolism and excretion of dronedarone was evaluated in CD-1 mice, Sprague-Dawley rats, New Zealand White rabbits, Beagles and Macaca fasicularis monkeys. There was also some examination of the effect of dronedarone on the CYP450 system. Oral administration studies were conducted using a suspension of drug in 0.6% methylcellulose or, for dogs, gelatin capsules. Intravenous administration studies with formulations of PEG400/sodium acetate buffer or mannitol/sodium phosphate were carried out in rats, dogs and macaques.

The oral bioavailability in rats and dogs is relatively low: 14-22%. The volume of distribution is large, 12 to 66 l/kg with a moderately high systemic clearance of 2-4 l/h/kg. The apparent terminal half-life after oral dosing is from 2-7 hours.

In all species studied, dronedarone was highly protein bound (>99%). The drug stays primarily in the plasma, not the red blood cells. There is wide tissue distribution (lung, liver, spleen, kidney, myocardium) including crossing the placental barrier and entering milk and bile. Radioactivity has also been identified in the brain, indicating that either drug and/or its metabolites cross the blood brain barrier. Low levels of radioactivity persisted after 336 hours in the liver, spleen and testis. Melanin binding was also demonstrated.

The drug is extensively metabolized and the sponsor's diagram of the proposed metabolic scheme can be found below. N-debutylation leads to SR35021, an active metabolite and the main circulating species in mice. A carboxylic acid derivative, SR90154, has limited pharmacologic activity and is one of the main circulating metabolites in rats and dogs.

The main route of excretion after both oral and intravenous administration is via the feces and via the bile in rat and dog. There is a minor amount excreted in the urine. Aliquots of urine treated with β-glucuronidase indicate that there is little if any conjugation of the metabolites.

2.6.4.10 Tables and figures to include comparative TK summary

PK parameters for SR33589B and SR35021 after single oral and IV doses

Species/route	Rat/po	Rat/iv	Dog/po	Dog/iv	Macaque/iv
Dose mg/kg	30	10	30	5	1
dronedarone					
Parameters					
Tmax (h)	4.0	Na	3.55	Na	Na
Cmax ng/ml	190-180	Na	241	Na	Na
AUC Ng.h/ml	1150-960	1740-1750	3360	2500	233
T1/2 h	6.4-6.6	6.1-12.5	3.8	3.4	1.9
Cl (l/hr/kg)	Na	4.4-2.3	Na	2.4	4.9
Vz (l/kg)	Na	38.5-66.2	Na	12.0	12.8
F%	22-18	Na	14	Na	Na
SR35021					
Tmax(h)	4.0	Na	Nd	Na	Na
Cmax ng/ml	52-30	Na	Blq	Na	Na
AUC ng.h/ml	nd	nd	nd	nd	nd

Metabolites in main toxicological species compared to metabolites in human plasma following oral administration of ^{14}C-dronedarone

Metabolite name	species				
	mouse	rat	rabbit	dog	human
Dronedarone	P12%, f	P,f,b	P4.4%,f	P19%,f	P10%,f
SR35021	P19%,f	P,f	P1.1%,u,f	P,f	P30%,u,f
SR90154	P9%,f	P,f,b	P5.5%,f	P31%,f	
Dronedarone derivatives					
Monohydroxy-SR35589	P17%,f	P,u	P8.4%,u,f	P17%,u,f	4.8%,u,f
Dihydroxy-SR35589	P4%,f	U,f		P15%,u,f	P5.8%u,f
Trihydroxy-SR35589	T			T	P0.7%,u,f
Monohydroxy-keto-SR35589			P21%,u,f		p1.1%,f
SR35021 derivatives					
Monohydroxy-SR35021	P3%,u,f	P,u,f,b	P1.8%,u,f	P11%,u,f	P4.7%,u,f
Dihydroxy-SR35021	P10%,u,f	U,f,b	P3.3%,u,f	P7%,u,f	P4.9%,u,f
Keto-SR35021	T	T		T	P0.9%,u,f
Monohydroxy-keto-SR35021	P7%,u,f	U,f,b	P19%,u,f	u	P2.5%,u,f

% of total radioactivity circulating in plasma at 2hours in mouse and dog, over 0-24 hours in rabbit and at 4 hours in human

b:bile, f:feces,p:plasma,u:urine, T: metabolite detected in trace amounts in at least one biological matrix

Because of the striking thrombotic effects in the intravenous toxicology studies, the metabolites seen after oral administration were compared with those seen after intravenous administration. I came to the conclusion that the parent drug has prothrombotic properties.

Male and female rats

After single oral administration	After single IV administration
SR90154 males 16-70% of total circulating radioactivity females 39-89% SR33589 males 11-45% of total plasma radioactivity females 4-26%	SR33589 was main circulating species up to the 10th hour after dosing

Dogs

Single oral administration	Single IV administration
O-propanoic acid SR33589 derivative: main circulating metabolite over 1st 10 hours (23-74% of all detected metabolites) SR33589 minor SR35021 minor Compound of unknown structure minor	SR33589 main circulating metabolite over 1st 10 hours (19-76% of all detected metabolites) SR35021 was not positively identified O-propanoic acid SR33589 derivative minor

Macaques

Single oral administration	Single IV administration
Not done	SR33589 62-100% of total radioactivity SR35021 minor SR90154 minor

2.6.5 PHARMACOKINETICS 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] Tables provided by the sponsor were of poorly scannable/transferable quality. Use of the sponsor's tables produced a "Compressed file virus" corruption of this review.

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

/s/

Elizabeth Hausner
3/3/2006 03:08:15 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
3/6/2006 08:15:18 AM
PHARMACOLOGIST

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: *Summary of Single Dose Toxicology*: The single dose toxicology studies were conducted in mice and rats. Death was not seen following single oral doses up to 2000 mg/kg in both sexes of mice and rats. Clinical signs associated with doses ≥1500 mg/kg , the lowest dose evaluated, were prostration, hematuria and ptyalism. Following bolus intravenous administration of 15-20 mg/kg, death was seen in both sexes of mice and rats. For both sexes and species, an LD50 for intravenous administration of SR33589B can be estimated to be between 15-20 mg/kg.

Summary of Repeat Dose Toxicology: The repeat dose oral administration studies were conducted in rats and dogs. Mice were used in the 3 month dose-ranging study and the 2 year carcinogenicity study. In dogs and rats, the same target organs of toxicity were identified: the gastrointestinal system and biliary tree, the kidney, the reproductive system of both sexes and the thyroid. The long term rodent students identified angiomatous changes in both mice and rats. Changes were also reported in the heart which included AV block in both species, decreased heart rate, increased PR interval and increased T wave amplitude (dogs). Increased heart weight was inconsistently reported. The effects on the heart and ECG are expected from the pharmacology. There is also a question whether the increases in heart weight may contribute to some of the ECG alterations.

Ptyalism was seen in both rats (≥30 mg/kg) and dogs (≥45 mg/kg). At doses of ≥70 mg/kg in rats, ptyalism was associated with histological changes of epithelial hyperplasia and hyperkeratosis of the esophagus and stomach. Erosive and ulcerative lesions were seen in the glandular stomach. Histolopathological findings in the liver included necrotic foci. Biliary fibrosis and hyperplasia were also reported. The histological findings from the esophagus and stomach are characteristic of what may be seen in response to an irritant substance. This is consistent with the irritant and prothrombotic effects seen in the intravenous administration studies. The histological findings are also to be expected from the variable clinical chemistry changes seen in serum triglyceride, cholesterol, AST and ALT. Dogs showed clinical signs of vomiting and diarrhea. Histological changes in the gastrointestinal system included necrotic foci in the liver. The pancreas showed inconsistent changes in both species including serous dedifferentiation. Sometimes the overall effects in the animals could be attributed to chronic sympathetic stimulation ("stress") as demonstrated in increased serum glucose, changes in circulating electrolytes, adrenal hyperplasia, splenic lymphoid atrophy and accelerated thymic involution. However, chronic sympathetic stimulation does not account for all reported changes. The single dose safety pharmacology studies are supportive of the changes reported in the toxicology studies and are not likely attributable to "stress".

Inconsistent hematologic changes were seen. Hemoconcentration was apparent in the two-week rodent study with an accompanying decrease in reticulocytes percent in both sexes. The absolute number of reticulocytes decreased in females(~0.5x) and increased in males (~4x). For the most part, hematology changes across studies were mild, consisting of decreases in RBC, PCV and Hb that while statistically significant were of questionable biological significance.

Effects upon the renal system were typically manifested in the serum chemistry and urinalysis without associated histological findings. Serum urea was increased in the 2 week rat study (160 mg/kg) in both sexes. In the 2 week rat study a dose-related increase in serum sodium and potassium was seen in both sexes (doses ≥30 mg/kg). In the 3 month study, increased serum urea, increased serum creatinine and increased serum sodium (≥5 mg/kg/day) were seen in both sexes and increased serum potassium in the females only (≥5 mg/kg). Serum creatinine and inorganic P were increased in the 6 month rat study (≥50 mg/kg).

Decreases in excreted electrolytes were seen in the 2-week rat study(160 mg/kg), the 2-week dog study (≥25 mg/kg), the 3-month dog study (decreased sodium excretion at 17.5 mg/kg, 60 mg/kg) and the 1-year

dog study. In the 1-year study urinary sodium and potassium were decreased at 45 mg/kg (HD) in the males. Urinary potassium was decreased in the drug-treated females in a non-dose related pattern. Urinary sodium was increased in the HD females days 86 and 177 but decreased day 363. Urinary creatinine excretion was decreased in the HDm and all groups of drug-treated females with a dose-response. Kidney weight was inconsistently increased, usually at the HD in both species.

Foamy macrophages were reported in various organs in rats. At doses ≥5 mg/kg (3 month study) foamy macrophages were reported in the lungs, tracheobronchial lymph nodes, popliteal lymph nodes and mesenteric lymph nodes. At doses ≥17.5 mg/kg (3 month study) foamy macrophages were also reported in the ileum and the femoral bone marrow. Electron microscopy indicated that the macrophages contained lipids. The sponsor then referred to the foamy macrophages as due to dyslipidosis.

Because of the similarity to amiodarone, effects upon the thyroid were given special consideration. Thyroid hormone levels were measured in several studies. Findings or trends from those studies are summarized below. It may be seen that effects do not necessarily follow a strict dose-response and are not always very profound.

14 day safety pharmacology study in rats

Effects of orally administered dronedarone and amiodarone on thyroid hormone levels

group	T4 (ng/ml)	T3 (ng/ml)	rT3 (pg/ml)	T4/T3 ratio
Control	54±5(10)	0.93±0.10(9)	60±13(9)	58±3(9)
SR33589B 50 mg/kg	56±12(10)	0.98±0.17(10)	61±22(10)	57±6(10)
SR33589B 100 mg/kg	49±9(9)	0.76±0.12(9)	63±17(9)	62±4(8)
SR33589B 150 mg/kg	35±9***(10)	0.76±0.18(10)	48±14(10)	46±6***(10)
Amiodarone 50 mg/kg	62±10(10)	0.94±0.10(8)	133±21***(10)	66±11(8)
Amiodarone 100 mg/kg	61±11(10)	0.81±0.15(10)	210±21***(9)	69±10**(10)
Amiodarone 150 mg/kg	50±9(10)	0.73±0.09**(10)	222±48***(10)	75±2***(10)

**p<0.01, ***p<0.001 compared to control group
(n)number of determinations (in duplicate for T4, T3, rT3 measurements)
[a] No detectable levels of SR33589B in 3 rats (<0.017 nmol/ml)
[b] No detectable levels of SR33589B in 1 rat (<0.017 nmol/ml

3 month rat tox

Summary of thyroid tests, day 98

	Male: dose mg/kg/day			
	0	5	17.5	60
TSH ng/ml	9.6±0.89	10.1±1.1	10.3±0.92	11.3±1.7
T3 nM	1.02±0.06	0.81±0.09	0.97±0.04	0.78±0.05
T4 nM	34.34±1.79	31.03±1.68	35.72±0.83	31.93±1.54
	Female			
TSH ng/ml	6.7±0.43	5.9±0.20	6.9±0.44	8.4±0.56
T3 nM	1.43±0.05	0.91±0.03***	0.96±0.04***	0.99±0.04***
T4 nM	35.66±2.47	34.15±1.07	31.89±1.37	34.52±1.17

***p<0.1%

6 month rat tox

Summary of Thyroid hormone changes

Dose mg/kg	T3 (nM)		T3 (nM)	
	M Day 185	M day 245	F Day 185	F Day 245
0	0.98±0.04	0.72±0.05	0.82±0.04	0.96±0.06
2	0.97±0.04		1.24±0.06***	
10	0.91±0.06		1.12±0.05***	
50	0.80±0.04*	0.62±0.035	0.82±0.06	0.66±0.05**

*<1.8%, **0.2%, ***0.1%

3 month oral toxicity in the dog

Summary of thyroid hormone effects

Males				
Dose in mg/kg	0	5	17.5	60
T3 nM day 89	1.48±0.151	1.21±0.011	1.36±0.084	0.93±0.077**
T4 nM day 89	36.84±3.569	26.64±1.303*	32.11±1.730	27.44±2.609

Summary of thyroid hormone effects

Females				
Dose in mg/kg	0	5	17.5	60
T3 nM day 89	1.60±0.105	1.44±0.192	1.27±0.246	0.64±0.097**
T4 nM day 89	43.18±4.103	37.69±5.273	40.27±4.242	24.47±1.942*

1 year oral tox in the dog

Summary of thyroid hormone effects

Males				
	Dose mg/kg			
	0	5	15	45
T3 nM day 365	1.34±0.062	1.35±0.070	1.02±0.075	0.72±0.055***
Females				
T3 nM day 365	1.32±0.057	1.12±0.139	1.11±0.086	0.74±0.028***

* 1.0%<p≤5.0%; **0.1%<p≤1.0%; ***p≤0.1%

From the 1-year dog study

Study No.: TXC0970 (Continued)

Dose (mg/kg/day)[a]	0		5		15		45	
No. of Animals on Study	**M**: 5	**F**: 5	**M**: 5	**F**: 5	**M**: 5	**F**: 5	**M**: 5	**F**: 5
Clinical Chemistry (Day 90, 181, 365) (continued)								
Inorganic phosphate (mM)[b] (Day 90)	NAD	1.39	NTRC	1.22	NTRC	1.23	NTRC	1.01*
Immunoglobulins G (mg/L)[c]								
Day 181	4140	NAD	3955	NTRC	4018	NTRC	8100**	NTRC
IgGs and IgMs (day 181, 365)	NA	NA	NTRC	NTRC	NTRC	NTRC	NTRC	NTRC
Hormone levels[d]								
T3 (mM)								
Day 90	1.24	1.20	1.22	1.15	1.17	1.17	**1.04**	**0.94***
Day 181	1.15	1.26	0.91	1.08	**0.83**	**1.09**	**0.95**	**1.05**
Day 365	1.34	1.32	1.35[b]	1.12	**1.02****	**1.11**	**0.72*****	**0.74*****
T3/T4 ratio								
Day –7	0.040	0.037	0.037	0.034	0.036	0.029**	0.034	0.027***
Day 90	0.039	0.035	0.039	0.031	0.034	0.029*	0.035	0.024***
Day 181	0.034	0.034	0.034	0.023	0.026	0.023	0.035	0.024
Day 365	0.051	0.039	0.051[b]	0.031	**0.035****	**0.029****	**0.027 *****	**0.022*****

Abbreviations: NTRC = No treatment related changes; NAD = no abnormality detected

Histopathology from the various rat toxicology studies typically report increased columnar thyroid follicular epithelium, generally accepted as indicative of hyperplasia. However, the 2-year carcinogenicity studies do not report any hyperplasia, adenoma or carcinoma of the thyroid gland.

Amiodarone, as an iodinated drug, inhibits in vivo the 5'-monodeiodination of T4 in the rat liver. Male and female Sprague-Dawley rats given 5,16 or 50 mg/kg/day of amiodarone orally in the 2 year carcinogenicity studies had an increased incidence of thyroid follicular cell hyperplasia, adenomas and carcinomas when compared with the control groups. The B6C3F1 mice who received the drug in the 2 year studies did not show an increase in thyroid follicular cell hyperplasia or tumors. In published literature, amiodarone has been reported to decrease T3 levels and increase T4 and rT3 levels.

A side-by-side comparison of the effects of amiodarone and dronedarone on 5'-monodeiodination of T4 might have been useful information. Other than the measurement of thyroid hormone levels, no further examination of dronedarone's effect on thyroid metabolism was made.

Another consideration is drug effect on the half-life of thyroid hormones. Half-life can be shortened or lengthened due to changes in metabolism as evidenced in hyperthyroidism and hypothyroidism (Goodman and Gilman, "Thyroid and Anti-thyroid Drugs"9th edition). Something that would have been very helpful in this NDA is a radiolabel (^{125}I-iodine) study to determine if there were any changes in the metabolism of the thyroid hormones. For example, if there was a more rapid conversion to rT3, a biologically inactive form, or if circulating half-lives were altered.

An unexplained phenomenon was alteration in weight of eyes and brain, seen inconsistently in several, but not all, studies in dogs and rats. This is summarized in the reviewer's table below. Because the weight of both these organs is protected, finding changes is a flag. The sponsor does not address these findings. Histological findings were not reported in association with the weight changes. Possible explanations include 1)collection techniques 2)weighing techniques 3) fluid (blood, CSF, intracellular fluid) content,

which bears upon collection technique 4) pharmacologic effect and 5) random chance. Variations in normal thyroid metabolism may also contribute to increased size of the eyes.

Reviewer's Summary of Eye and Brain Weights

2 week rat oral toxicity study				
Doses mg/kg	0	30	70	160
Males: eyes g/kg	0.778±0.023	0.845±0.029	0.803±0.04	1.141±0.07***
Males : brain g/kg	5.96±0.17			8.08±0.41***
Females: eyes g/kg	1.149±0.045	1.038±0.027	1.027±0.047	1.443±0.082***
Females : brain g/kg	8.19±0.160	7.69±0.118	7.41±0.142	9.78±0.202***
3 month rat				
Doses mg/kg				
Males : Eyes g/kg	0.662	0.720*	0.672	0.685
Females : Eyes g/kg	1.010±0.03	0.994±0.02	0.977±0.02	1.008±0.03
6 month rat				
Doses mg/kg	**0**	**2**	**10**	**50**
Males: eyes g/kg	0.647±0.014	0.670±0.018	0.652±0.021	0.700±0.027
Males : brain g/kg	3.77±0.080	3.75±0.091	3.79±0.111	3.98±0.169
Females: eyes g/kg	1.092±0.026	1.168±0.031	1.101±0.019	1.075±0.030
Females : brain g/kg	6.30±0.148	6.58±0.146	6.37±0.124	6.16±0.165
2 week mouse study				
Doses mg/kg	0	450	600	
Males : brain g/kg	0.5005±0.014	0.4942±0.015	0.4720±0.016	
Females : brain g/kg	0.4983±0.014	0.4980±0.013	0.4843±0.018	
Eye data not present in report				
3 month mouse study				
Doses mg/kg	**0**	**50**	**150**	**450**
Males : brain g/kg	0.5016±0.008	0.5125±0.009	0.4932±0.009	0.4796±0.011
Females : brain g/kg	0.5524±0.010	0.5327±0.009	0.5460±0.008	0.5459±0.009
Eye data not present in report				

Reviewer's Summary of Normalized Eye and Brain Weights continued

Two week dog study				
Doses used	**0**	**25**	**60**	**140**
Males: eyes g/kg	1.047±0.10	1.026±0.01	1.192±0.01	1.008±0.04
Females: eyes g/kg	1.154±0.113	1.229±0.023	1.080±0.09	1.188±0.08
Males: brain g/kg	6.57±0.83	6.99±0.10	7.39±0.20	6.77±0.22
Females: brain g/kg	7.05±0.45	7.74±0.07	7.57±0.74	8.38±0.87
3 month dog study				
Doses used				
Males: eyes g/kg	1.1	1.11	1.002	1.265
Females: eyes g/kg	1.01	1.156	1.081	1.324*
Males: brain g/kg	7.27	7.5	7.3	7.87
Females: brain g/kg	6.45	7.36	7.43	9.02
1 year dog study				
Doses used				
Males: Brain g/kg	5.72±0.485	5.91±0.387	6.22±0.359	7.6±0.449*
Females: Brain g/kg	6.44±0.349	6.35±0.442	6.46 ±0.428	7.69±0.413

Genetic toxicology:

Dronedarone has been tested in the Ames assay, the mouse lymphoma, HPRT in Chinese hamster V79 cells, unscheduled DNA synthesis (in vitro) and a human lymphocytogenetic study.
The sponsor dismissed the positive results in the HPRT Chinese hamster cell assay as being within the range of historical controls.

Study #, Assay	Concentrations	Results
CEL0593 Ames assay	Preliminary: 94, 469, 938, 2345,4690µg/plate Genotox: 4.7, 9.4, 47, 94, 235 µg/plate	No increase in revertants
CEL0709 Ames assay	Preliminary (T98): 4.7, 9.4, 47, 94, 235, 469 µg/plate Genotox: 9.4, 23, 47, 94, 164 µg/plate	TA98 +S9 showed a repeatable increase (NS) in revertants. A full dose response was not shown due to the level of toxicity at the concentrations tested.
LYM0125 Mouse lymphoma	3hr ±S9: 9.37, 18.75,37.5, 75, 150, 300 µg/ml 24 hr-S9: 0.1, 0.25, 0.5, 1,2,4 and 5 µg/ml 3hr-S9: 2.5, 5, 7.5, 10, 12.5, 15 µg/ml 3hr+S9: 10, 15, 20, 25, 30, 35 µg/ml 24 –S9: 0.5, 1,1.5, 2,2.5, 3 µg/ml 3hr+S9: 17.5, 20, 22.5, 25, 27.5, 30µg/ml	No indication of increased mutation frequency in the data as presented.
685-3-008 HPRT in Chinese hamster V79 fibroblasts	1st±S9: 2.5, 5,10,25,50, 100 µg/ml 2nd –S9: 1,2.5,5,10, 17.5 µg/ml 2nd+S9: 5,10,17.5,25,37.5 µg/ml	With S9, statistically signif-icant increases in mutants were seen in 3 repeat assays
DNA0001 in vitro DNA repair assay in primary culture rat hepatocytes	1, 2.5, 5, 10, 25 µg/ml	No evidence of unscheduled DNA synthesis in the data as presented
CEL0536 in vitro DNA repair assay in primary culture rat hepatocytes	Cytotoxicity study: 1,5,10,50,100, 250 µg/ml DNA repair study: 1,5,10,25 µg/ml	No evidence of unscheduled DNA synthesis in the data as presented
MAF0018 Lymphocyte cytogenetic study	Prelim ±S9: 25, 50, 100, 200, 600 µg/ml Main –S9: 1.25, 2.5, 5,10,15,20 µg/ml Main+S9: 6.25,12.5, 25,35,40,45 µg/ml	
MUT0046 Mouse micronucleus	Single oral dose of 2000 mg/kg	No evidence of increased micronuclei as presented

Carcinogenicity: The Executive CAC found the following to be drug-related: histiocytic sarcomas in male mice, mammary adenocarcinomas in female mice and the hemangiomas in male rats. The full details may be found in the minutes of the Exec CAC meeting (Appendix C in NDA_partIV_Appendices), the Pharmacology/Toxicology review of the studies (Appendix B NDA_partIV_Appendices) and the CDER statistical review by Jialu Zhang, Ph.D.

Carcinogenicity study finding	Sponsor's response to CA study findings
Vasoproliferative lesions in mesenteric lymph nodes	'…the incidence of hemangioma was much higher than that described for other SD rats in the literature. It thus seems that the strain used in our study exhibited an increased susceptibility to…this lesion, and that …dronedarone results in an increase of this spontaneous pathology." P 35 of Nonclinical Overview
Histiocytic sarcoma	"Changes were observed at a low incidence, and the relevance of such marginal findings remains equivocal since this could be the result of normal variation or represent a weak carcinogenic response;… No chemical products are known to induce histiocytic sarcoma in rats; in mice, 3 compounds are known to cause such changes…There was no increase in the frequency of other lymphoreticular tumors in both carcinogenicity studies." P 35 of Nonclinical Overview
Mammary adenoma and adenocanthoma in female mice	"Changes were not associated with target organ toxicity" "Tumors were limited to 1 sex, species and HD group…The proven prolactin-dependent pathogenesis of rodent tumors(24) is different from that in humans…" P 36 of Nonclinical Overview

The sponsor submitted with the NDA a narrative summary from a "Scientific Advisory Panel" (SAP) provided by Experimental Pathology Laboratories, a contract pathology laboratory. The SAP gave their opinion on the relevance to humans. They concluded that dronedarone did not pose a carcinogenic risk for humans.

My response to the arguments provided:
Vasoproliferative lesions: An increase in spontaneous lesions is considered a positive carcinogenicity finding.

Histiocytic sarcoma: The foundation of the sponsor's argument is a 1990 report (Squire) stating that at that time, no chemical had been shown to increase the incidence of histiocytic sarcoma in rats. However, 3 NTP reports (1993, 1996 and 1997) did demonstrate that there were 3 chemicals causing increases in histiocytic sarcoma in mice. Why are these relevant arguments and how does a 16-year old report have any relationship to the current drug? The report states that histiocytic sarcomas are rare in mice until 12 months of age and cites historical control data that was published by Haseman et al in 1986. Given the change in laboratory animal genetics and husbandry conditions, we may question the relevance (historical controls are usually requested to be the most recent possible) of these findings to a contemporary study. The report notes that "Except for receptor mediated (hormonal) carcinogens, the SAP knows of no nongenotoxic chemicals that have been shown to cause mesenchymal neoplasms when administered systemically, other than tetrafluoroethylene which was not extensively tested for genotoxicity by the NTP." This seems to be saying that hormonal mechanisms have been shown to cause increased incidence of histiocytic sarcomas. The SAP comes to the conclusion that "The relevance of histiocytic sarcoma in mice to humans is unknown since an analogous neoplasm is extremely rare in humans." The final decision that the finding did not represent a significant risk to humans was based upon the postulated margin of safety at the highest dose and the recommended therapeutic dose.

Mammary gland adenocarcinoma in female mice: The sponsor states that "In the absence of concurrent changes in ovaries, pituitary gland or adrenal gland, the mechanism leading to the increased numbers of mammary tumors in the current study is unknown. …The morphology of the neoplasms is species specific and dissimilar to mammary tumors observed in humans. Mammary gland tumors are generally considered to be associated with elevated prolactin levels, In humans, mammary gland neoplasms are not related to prolactin levels." Even though it is stated that the mechanism behind the tumors seen is unknown, an assumption was made that it must be

a) prolactin
b) unrelated to humans

I find the assumptions in the absence of mechanistic data to be disturbing. In the two studies where prolactin was measured, it was unchanged in one study and in the other study showed a **dose-related decrease.** There were no measurements of prolactin in the carcinogenicity study.

I find it very disturbing that the view of prolactin being unrelated to human mammary carcinogenesis was presented in isolation by the SAP. A 5 minute search using PubMed and the terms

"mammary neoplasia" AND prolactin
"mammary adenoma" AND prolactin
"mammary carcinoma" AND prolactin

produced over 100 references and a substantial body of research making a case for the role of prolactin(PRL) in 1)higher risk of human breast cancer independent of estradiol/other sex hormone levels 2)a decreased time to human breast cancer recurrence 3) an increase in human metastatic disease and 4) increased mortality. A not-exhaustive examination of the literature showed a few recent publications claiming that prolactin is luteotrophic in rodents but not humans and the mechanism of prolactin-induced rodent mammary carcinogenesis is argued to not be relevant to humans. But these publications appear to ignore the epidemiologic, clinical, molecular and transgenic research regarding prolactin and human mammary carcinogenesis. An exhaustive examination of this subject is beyond the scope of this review. However, there are several reviews in the scientific literature making a case for rodent mammary tumors being significant for human carcinogenesis and again I am disturbed that the Scientific Advisory Panel is either unaware of this research or chooses not to mention it. If they find the research flawed to the point where it is unsupportive of the argument that PRL is involved in human mammary carcinogenesis, a statement to that effect would have been appropriate in the interests of fairness and full disclosure.

Because many of the drugs prescribed for psychoses and schizophrenia cause increased prolactin levels, I asked Lois Freed, Ph.D., Supervisory Pharmacologist for the Division of Neuropharmacological Drugs how that Division handles drugs where mammary tumors are seen in the carcinogenicity studies. Dr Freed replied that if a sponsor wishes to claim that the tumors are prolactin based, they are required to show increased circulating

prolactin levels. These increases typically are not subtle but may be 50 to 100 fold over normal levels. Also, the risk-to-benefit ratio is part of the consideration for approval where prolactin-related mammary tumors have been reported.

One final comment on the carcinogenesis findings. A hormonal mechanism could explain all 3 of the tumor findings. The sponsor seems to be arguing for 3 different tumors caused by 3 different mechanisms of action. While this is possible, it seems a lower probability than one drug with one mechanism.

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Reproductive toxicology: The sponsor has already stated in their proposed labeling (and I do not disagree with this) that dronedarone is teratogenic in rats. In a preliminary study, no maternal toxicity was noted in dams at doses of 10, 20, 40 and 80 mg/kg/day with the exception of a mild decrease in body weight gain from GD6-GD10. Maternal toxicity was seen at the HD. Two dams that received 160 mg/kg/day died while the food consumption and body weight gain was greatly decreased. There were skeletal, visceral

and external malformations in 17 out of 18 fetuses. At 80 mg/kg/day, with no maternal toxicity, skeletal malformations similar to those seen at 160 mg/kg were reported for 15 out of 48 (~30%) of the fetuses. The main rat embryo-fetal toxicity study used doses of 0,10, 30 and 100 mg/kg/day. The HD animals gained 57% less body weight throughout the treatment period. No maternal toxicity was seen in the LD and MD groups. In the HD group, 198 out of 221 fetuses had abnormalities that affected external morphology, all major organ systems and the skeleton. While there were no reported anomalies in the MD group, there were reports of unilateral anophthalmia and unilateral microphthalmia in the LD group. Were these coincidental? While the sponsor did not consider the drug to be teratogenic in rabbits, skeletal abnormalities were seen at all doses in the definitive study, including the LD (20 mg/kg) with no maternal toxicity. Two fetuses from different litters of the LD, were grossly abnormal. One had a proboscis, microcephaly, both ears pointing forwards, cyclopia, agenesis of the lower jaw and mouth and an enlarged and misshapen larynx. The other had a large, misshapen larynx, central displacement of both eyes with partially open eyelids, enlarged snout with agenesis of nares and agenesis of lower jaw with no mouth. The sponsor felt that these were not drug-related. While I am not sure of this, there is agreement between reviewer and sponsor that the drug is teratogenic.

Another issue that appeared was an effect upon female cyclicity. This manifested in a number of toxicology studies with the drug-treated females paused in diestrus. In the reproductive toxicology studies where multiple analyses were made, prolonged cycles, irregular cycles and acyclicity were seen in the drug-treated females. Effects upon the weight of the uterus and ovaries were also seen in several studies. An interesting point is that these effects were reported in studies where the females were exposed to drug for ≤3 months. These effects are summarized in the reviewer's tables below.

Female cyclicity data

2 week rat oral toxicity				
Doses mg/kg/day	0	30	70	160
Estrus cycle phase:				
Diestrus	1	5	4	8
Proestrus	2	2	0	0
Estrus	0	0	1	0
Metestrus	7	3	5	0
3 month oral toxicity				
Doses mg/kg/day	0	5	17.5	60
Estrus cycle phase:				
Diestrus	4	10	8	10
Proestrus	1	1	2	1
Estrus	1	2	3	1
Metestrus	9	2	2	3
6 month oral toxicity study in rats : no data provided				

Female Cyclicity Summary Continued

Segment I Reproductive Toxicity Study				
Doses mg/kg/day	0	10	30	100
FER250: Estrous Cycles Arithmetic number (percent)				
Regular 4 or 5 day cycle	6(100)	4(67)	5(83)	6(100)
Irregular cycle [a]	0	1(17)	1(17)	0
Acyclic [b]	0	1(17)	0	0
FER0297				
Doses mg/kg/day	0	10	30	100
4 day estrous cycle	20(91)	18(82)	10(45)[b]	11(50) [b]
4/5 day estrous cycle	1(5)	2(9)	4(18)	9(41)[b]
5 day estrous cycle	0	0	4(18)	0
Irregular λ	1(5)	0	0	1(5)
Acyclicψ	0	2(9)	4(18)	1(5)

[a] At least one cycle of two, 3, or six-10 days
[b] At least 10 days without estrous
λ at least one cycle of 2,3 or 6-10 days
ψ at least 10 days without estrous

Female reproductive organ weight summary

2 week oral toxicity study in rats				
Doses mg/kg	0	30	70	160
Pituitary mg/kg	66.2±2.54			74.2±4.31
Uterus g/kg	2.46±0.24	2.26±0.24	1.97±0.15	1.49±0.12*
Ovaries mg/kg	440±30	416±19	449±18	451±34
3 month oral toxicity study in rats				
Dose mg/kg	0	5	17.5	60
Uterus g/kg	2.252±0.17	2.12±0.17	2.397±0.24	1.84
Ovary mg/kg	298±19.5	289±16.5	303±11.1	334±12.5
6 month oral toxicity study in rats				
Dose mg/kg	0	2	10	50
Uterus day 191 Day 246	2.474±0.16 2.322±0.21	2.686±0.20	2.226±0.21	1.973±0.12 2.25±0.11
Ovary day 191 Day 246	324±14.7 346±38.2	312±12.2	345±12.1	347±16.4 265±39.1
Dog 2 week oral toxicity study				
Dose mg/kg	0	25	60	140
Uterus g/kg	0.437±0.13	0.572±0.10	1.375±0.14	1.876±0.55
Ovaries mg/kg	102±8.4	108±16.1	161±9.6*	161±7.9*
Dog 3 month oral toxicity study				
Dose mg/kg	0	5	17.5	60
Uterus g/kg	0.359±0.0413	0.493±0.0540	0.880±0.3553	0.407±0.0661
Dog 2 week IV toxicity study				
Uterus g/kg	0.46±0.19	0.971±0.54	1.29±0.53	1.26±0.56
Ovaries (mg/kg)	66±12	114±37	121±32	156±54

Special toxicology: Special toxicology covered 1) hemolytic potential in vitro 2) phototoxicity and photoallergy and 3)immunological effects
Hemolytic potential was assessed in baboon and human blood. In the human study (HEM0015), after 30 minutes of incubating drug and blood, the 0.5% (LD), 0.9%(MD) and 1.4% (HD of undiluted formulation) hemolysis seen with the drug slightly exceeded that seen with the vehicle (0.2%). After 2 hours of incubation, only the HD produced more hemolysis (5.1%) than did the vehicle (3.3%). None of the in vitro hemolysis studies provided standard curves to allow the reader to assess the range of measurement of the assay.

The sponsor decided not to pursue the intravenous indication for dronedarone. For completeness, the majority of intravenous administration toxicology studies were reviewed. The sponsor identified the liver as the target organ of toxicity, poor local tolerability and venous thrombosis and phlebitis as secondary to the intravenous administration. I agree with the sponsor's assessments. Poor tolerability was seen in rats, dogs and macaques, the three species in which intravenous dosing was tried. Consistent with oral dosing, changes were seen in the serum and excreted electrolytes. In the dog studies, changes were seen in the weight of the female reproductive tract (see above). In the macaques, dose-dependency for the thrombotic effects was reported as lacking. Increased PR and QT as well as AV block were reported in the dogs and macaques. The macaques seemed to be very sensitive to the adverse effects. In the 2-week IV dosing study, dose-related increases in tissue irritation were seen at ≥1 mg/kg. The damage included profound gross and microscopic changes of hemorrhage, inflammation, thrombosis and muscle necrosis. Not presented in the summary tables but mentioned in the text were reports of changes in the veins of the axilla, cases of vascular rupture, segmental venous necrosis and muscular and dermal hemorrhagic infiltration. There was further mention of local opportunistic infections, a few cases of widespread necrosis of the crural muscle with inflammatory, septic, edematous and hemorrhagic changes sometimes associated.

Three studies were conducted in guinea pigs to examine the potential for phototoxicity, photoallergy and photosensitization associated with tissue storage. After one dose of drug and UVA +UVB irradiation, a phototoxic response was noted in 8/10 animals at 100 mg/kg and in 8/10 animals at 200 mg/kg. The reactions were graded 1-2 on a scale of 0-4 with 0 being no reaction. The score 2 was listed as well-defined erythema, pale pink. The studies did not provide evidence of photoallergy.

Dronedarone's structural similarity to amiodarone, which has a black box warning for pulmonary hypersensitivity pneumonitis or interstitial alveolar pneumonitis, may well

have been the impetus for the immunotoxicology studies. In the Toxicology Summary, p.230/465 the sponsor describes some of the changes seen as classical health deterioration, to include: lymphoid atrophy of the thymus (involution) or lymph nodes, fatty involution of the bone marrow and necrosis or hemorrhage in the adrenal cortex. Dyslipidosis was characterized by foamy macrophage infiltration of the thymus, spleen and lymph nodes as well as the non-lymphoid tissues of liver, lung and intestine. These signs may also be taken as potential immune system effects.

According to the Guidance on Immunotoxicology Evaluation of Investigational New Drugs, evaluation of potential immune system effects may include serum globulin levels (immunosuppresion), hematology differential, immune system-related organ weights and their histopathology. The most widely accepted method of experimental evaluation of immune system function is the effect on immune response to a T-cell dependent immunogen. This was in fact the method the sponsor used in the 4-week oral toxicity study in the rat.

Assessment of potential immunotoxicity included A) 3-month and 6-month rat toxicity studies: measuring IgG and IgM concentrations and examining lymphocyte subpopulations (6-month study only). B) 3-month and 1-year dog toxicity studies: IgG and IgM concentrations, lymphocyte subpopulations and lymphoblast transformation tests (1-year study only). A 4-week immunotoxicity study using male rats at doses of 2,10 and 50 mg/kg/day (the doses from the 6-month study) evaluated bone marrow cellularity, hematology, selected lymphocyte subpopulations and functional assessment of IgG and IgM blood levels in response to stimulation from keyhole limpet hemocyanin (KLH). There was no apparent difference in primary or secondary response as measured by anti-KLH titers. The lymphoblastic transformation study did show decreases in cpm of ^{3}H-thymidine with corresponding decreases in the standard error of the mean for the measurements. The variability of the various assays was discussed with Ken Hastings, Ph.D. of the Immunotoxicity Committee. Dr Hastings confirmed that these assays typically show a great deal of variability. It is further possible to obscure positive results by using a positive control that is extremely potent. However, in the lymphoblastic transformation assay as an example, a decrease in cpm with a decrease in sem may in fact indicate significant results. A summary of the results from the various immunotoxicity assays was shared with Dr Hastings (email communication). His feeling was that the results from the 3 month rat study were possibly indicative of a subclinical infection. The effects overall were mild enough that issue was not one of significance.
The data specifically pertaining to the immunotoxicology is summarized below (the same material that was provided to Dr Hastings).

3 month rat study

Summary of lymphocyte populations: mean %±SEM and (mean absolute (10^3/mm^3))

	Males: dose (mg/kg/day)			
	0	5	17.5	60
CD4+	44.7±5.5	52.8±4.2	51.7±4.9	52.9±3.7
CD8+	3.5±0.9 (0.26)	4.6±0.9 (0.28)	6.3±1.1 (0.39)	6.2±1.0 (0.42)
B	27.0±2.8(2.07)	19.8±1.3(1.21)	18.7±1.2*(1.19)	23.5±1.5 (1.54)
Females (mg/kg/day)				
CD4+	62.6±1.9	62±2.7	62.8±2.8	61.3±3.5
CD8+	5.5±0.8	6.8±1.1	7.4±1.1	7.6±1.54
B	18.9±0.7	18.7±1.5	16.3±0.7	19.4±2.6

IgM was increased at the HD for males and in females showed a dose-related increase.

Summary of Immunoglobulin findings day 103

	Males: dose (mg/kg/day)			
	0	5	60	160
IgM mg/l	1170±52	1162±34	1166±39	1319±49
	Females			
IgM mg/l	1150±29	1191±49	1268±56	1500±35***

***p<0.1%

Rat 6 month toxicity study

	IgG mg/l		IgM mg/l	
	males	females	males	females
vehicle	10242±593	11027±645	1199±47	1213±41
10 mg/kg	10211±899	10557±1159	1296±44	1253±41
30 mg/kg	13318±1150*	10196±855	1390±41*	1474±44***

*1.0%<P<5.0%, ***P≤0.01%

There were no apparent findings of significance in either the lymphocyte subpopulation analysis or the bone marrow cellularity.

Lymphocyte subpopulations (%) day 185

MALE	CD4+	CD8+	B	Total
Levene	8.6% NS	>10% NS	6.8% NS	>10% NS
1-ANOVA	0.4% **	>10% NS	>10% NS	>10% NS
Group 0 : Vehicle				
n = 30	(5)	(5)	(5)	(5)
mean	23.6	7.8	42.5	73.9
SEM	2.26	0.85	6.16	6.47
Group 1 : SR 33589B 2 mg/kg				
n = 20	(5)	(5)	(5)	(5)
mean	40.0	10.6	34.1	84.7
SEM	3.33	1.48	1.28	3.58
Test/group 0	<0.1%***			
Group 2 : SR 33589B 10 mg/kg				
n = 20	(4)	(4)	(4)	(4)
mean	34.1	9.4	38.3	81.7
SEM	3.65	1.19	4.08	2.63
Test/group 0	5.3% NS			
Group 3 : SR 33589B 50 mg/kg				
n = 30	(5)	(5)	(4)	(4)
mean	32.3	10.1	40.1	82.9
SEM	1.24	0.89	3.05	3.27
Test/group 0	9.7% NS			

Lymphocyte subpopulations (%) day 185 (Cont'd)

FEMALE	CD4+	CD8+	B	Total
Levene	>10% NS	>10% NS	8.6% NS	3.3% *
1-ANOVA	>10% NS	>10% NS	>10% NS	---
Group 0 : Vehicle				
n = 30	(5)	(5)	(5)	(5)
mean	51.3	12.6	26.2	90.0
SEM	3.00	1.27	0.95	3.09
Group 1 : SR 33589B 2 mg/kg				
n = 20	(5)	(5)	(5)	(5)
mean	48.6	10.5	23.5	82.6
SEM	3.51	1.65	3.34	1.96
Test/group 0				7.8% NS
Group 2 : SR 33589B 10 mg/kg				
n = 20	(5)	(5)	(5)	(5)
mean	45.2	9.0	24.5	78.7
SEM	3.16	1.37	3.04	1.83
Test/group 0				1.4% *
Group 3 : SR 33589B 50 mg/kg				
n = 30	(4)	(4)	(4)	(4)
mean	50.3	11.1	25.2	86.5
SEM	1.76	1.46	1.80	1.14
Test/group 0				>10% NS

Table (5.5.3) 2.
Microscopy: lymph nodes - End of treatment (6 months)
(mesenteric, popliteal, retromandibular and tracheobronchial lymph nodes)

Group	0		1		2		3	
Test compound	Vehicle		SR33589B		SR33589B		SR33589B	
Dose (mg/kg/day)	-		2		10		50	
Sex	M	F	M	F	M	F	M	F
No. of animals: end of study	20	20	20	20	19	20	20	19
sacr. during study					1			1
Mesenteric lymph node								
- Macrophage infiltration								
very slight	4	10	8	8	-/4	6	1	-/7
slight	7	5	7	7	-/7	10	12	1/7
moderate	1	1	-	1	-/2	-	2	-/2
Popliteal lymph nodes								
- Macrophage infiltration								
very slight	3	5	3	5	-/6	7	2	-/7
slight	4	-	1	3	1/1	4	3	1/2
moderate	-	-	-	-	-/-	-	3	-/-
Retromandibular lymph node (examined on opportunity)								
- Macrophage infiltration								
very slight or slight	3	9	-	-	-/-	-	2	1/6
moderate or marked	1	1	-	-	-/-	-	3	-/1
Tracheobronchial lymph node								
- Very slight or slight medullary plasmocytosis	2	-	-	-	-/1	1	3	-/3

3 month dog study

Table (5.4.2) 1.
Lymphocyte subpopulations (%) day 85

MALE	T	B
Levene	2.6% *	>10% NS
1-ANOVA	---	>10% NS
Group 0 : EMPTY CAPSULE		
n = 4		
mean	76.7	12.4
SEM	1.60	2.33
Group 1 : SR 33589B 5 mg/kg		
n = 4		
mean	79.0	11.1
SEM	0.89	1.32
Test/group 0	>10% NS	
Group 2 : SR 33589B 17.5 mg/kg		
n = 4		
mean	74.4	10.8
SEM	7.98	3.17
Test/group 0	>10% NS	
Group 3 : SR 33589B 60 mg/kg		
n = 4		
mean	81.5	9.1
SEM	2.43	0.98
Test/group 0	>10% NS	

Table (5.4.2) 1
Lymphocyte subpopulations (%) day 85 (Con

FEMALE	T	B
Levene	>10% NS	>10% NS
1-ANOVA	>10% NS	>10% NS
Group 0 : EMPTY CAPSULE		
n = 4		
mean	79.9	10.6
SEM	2.31	1.67
Group 1 : SR 33589B 5 mg/kg		
n = 4		
mean	81.5	11.2
SEM	2.54	2.56
Group 2 : SR 33589B 17.5 mg/kg		
n = 4	(3)	(3)
mean	80.7	10.9
SEM	1.62	1.72
Group 3 : SR 33589B 60 mg/kg		
n = 4		
mean	84.1	8.0
SEM	2.84	2.71

The absolute numbers of lymphocytes do not show any consistent pattern and are not shown here.

Immunoglobulins : Class day 89

MALE	IgG mg/l	IgM mg/l
Levene	>10% NS	>10% NS
1-ANOVA	>10% NS	>10% NS
Group 0 : EMPTY CAPSULE		
n = 4		
mean	24500	1123
SEM	1936.5	248.7
Group 1 : SR 33589B 5 mg/kg		
n = 4		
mean	23500	1010
SEM	4092.7	149.8
Group 2 : SR 33589B 17.5 mg/kg		
n = 4		
mean	17375	945
SEM	1477.3	121.8
Group 3 : SR 33589B 60 mg/kg		
n = 4		
mean	20125	1215
SEM	2065.3	205.5

Table (5.4.3) 21
Immunoglobulins : Class day 89 (Cont'd)

FEMALE	IgG mg/l	IgM mg/l
Levene	>10% NS	>10% NS
1-ANOVA	>10% NS	>10% NS
Group 0 : EMPTY CAPSULE		
n = 4		
mean	16000	905
SEM	1779.5	119.5
Group 1 : SR 33589B 5 mg/kg		
n = 4		
mean	18625	1033
SEM	1313.0	87.7
Group 2 : SR 33589B 17.5 mg/kg		
n = 4		
mean	16250	838
SEM	1701.7	106.2
Group 3 : SR 33589B 60 mg/kg		
n = 4		
mean	17125	1010
SEM	1663.0	163.9

One year dog study

Summary of Immunoglobulin levels (Mean ±SEM)

Males				
Dose mg/kg	0	5	15	45
IgG mg/l day 181	4140±90	3955±112	4018±126	8100±653**
IgG mg/l day 365	11822±2994	10418±3014	8298±2470	5612±322
IgM mg/l day 181	1165±156	805±117	840±80	884±119
Females				
IgG mg/l day 181	4342±184	4112±85	4744±666	6392±1614
IgG mg/l day 365	5726±993	6100±1073	5400±1678	4344±245
IgM mg/l day 181	1303±258	885±117	1090±246	1119±260

*1.0%<p≤5.0%; **0.1%<p≤1.0%; ***p≤0.1%

Lymphoblastic transformation test: PHA (cpm) day 359 in males

Dose group mg/kg	Without mitogen	Conc. 1	Conc. 2	Conc. 3
Empty capsule	274±54	12834±3308	14479±4869	15634±6456
5	266±16	12077±2375	14071±2897	15614±3172
15	197±13	7793±1700	10161±2810	9963±2887
45	296±44	8001±1936	9340±2289	9858±3397
PWM: day 184				
Empty capsule	239±36	6322±2093	6985±2065	9670±2600
5	217±32	7541±1465	7757±1702	10316±2317
15	259±37	5377±680	5258±700	7648±1100
45	294±44	5263±728	5663±614	8109±492
PWM: day 359				
Empty capsule	330±50	6468±2747	6203±2250	9010±3722
5	363±43	7514±1400	9113±2368	11573±2641
15	244±40	4417±990	4891±1122	6904±1331
45	313±22	4294±1247	4686±1099	6638±1312

Lymphoblastic transformation test: PHA (cpm) day 359 in females

Dose group mg/kg	Without mitogen	Conc. 1	Conc. 2	Conc. 3
Empty capsule	385±121	10012±1739	13935±4020	14612±5150
5	213±12	6958±1022	8120±1356	8492±2154
15	300±46	7734±1879	10607±2978	13218±4518
45	268±31	8814±1255	12813±1592	11865±1874
PWM: day 184				
Empty capsule	159±18	4655±1448	5266±1671	7526±2329
45	272±31	3736±463	3781±490	5054±1052
PWM: day 359				
Empty capsule	305±39	6826±2544	7164±2377	9809±3359
45	242±24	5070±945	4815±661	6398±613

A 4-week oral immunotoxicity study in the rat (started August 25, 2005)

Table (3.4.4) 1 - IMM0044: anti-KLH IgM- and IgG-producing cells - Mean values

Group	1	2	3	4	5
Dose (mg/kg/day)	0	2	10	50	Cyclophosphamide
No. of animals/group	10	10	10	10	10
Anti-KLH IgM blood levels					
Primary response					
Titer log	3.23	3.05	3.32	2.93	1.90***
Variation	0	– 6%	+ 3%	– 9%	– 41%
Secondary response					
Titer log	3.41	3.11	3.17	3.29	1.96***
Variation	0	– 9%	– 7%	– 4%	– 43%
Anti-KLH IgG blood levels					
Primary response					
Titer log	3.05	3.20	2.96	2.78	1.53***
Variation	0	+ 5%	– 3%	– 9%	– 50%
Secondary response					
Titer log	3.44	3.32	3.29	3.35	2.68***
Variation	0	– 3%	– 4%	– 3%	– 26%

*** = $p < 0.001$ when compared to controls

Table (6.4) 6 - Lymphocyte subpopulations (%) day 28

MALE	CD4+	CD8+	B	NK
Levene	>10% NS	9.3% NS	>10% NS	0.9% **
1-ANOVA	>10% NS	<0.1%***	<0.1%***	---
Group 1 : VEHICLE				
n = 10				
mean	**35.7**	**17.1**	**39.8**	**3.9**
SEM	2.30	1.03	3.69	0.19
Group 2 : SR 33589B 2 mg/kg				
n = 10				
mean	**37.7**	**19.5**	**33.8**	**4.4**
SEM	1.36	0.69	1.84	0.45
Test/group 1		>10% NS	>10% NS	>10% NS
Group 3 : SR 33589B 10 mg/kg				
n = 10				
mean	**34.0**	**18.2**	**42.9**	**4.5**
SEM	1.39	1.60	1.84	0.67
Test/group 1		>10% NS	>10% NS	>10% NS
Group 4 : SR 33589B 50 mg/kg				
n = 10				
mean	**35.8**	**19.2**	**39.0**	**5.9**
SEM	2.32	0.88	1.98	0.81
Test/group 1		>10% NS	>10% NS	4.0% *
Group 5 : CP 4.5 mg/kg				
n = 10				
mean	**39.8**	**34.8**	**19.7**	**4.8**
SEM	1.46	1.33	1.98	0.49
Test/group 1		<0.1%***	<0.1%***	>10% NS

Table (6.4) 7 - Lymphocyte subpopulations (109/L) day 28**

MALE	CD4+	CD8+	B	NK
Levene	6.7% NS	0.2% **	4.7% *	<0.1%***
1-ANOVA	<0.1%***	---	---	---
Group 1 : VEHICLE				
n = 10				
mean	**3.62**	**1.74**	**4.13**	**0.40**
SEM	0.261	0.134	0.549	0.020
Group 2 : SR 33589B 2 mg/kg				
n = 10				
mean	**3.61**	**1.85**	**3.24**	**0.42**
SEM	0.324	0.127	0.337	0.044
Test/group 1	>10% NS	>10% NS	>10% NS	>10% NS
Group 3 : SR 33589B 10 mg/kg				
n = 10				
mean	**3.41**	**1.86**	**4.28**	**0.47**
SEM	0.235	0.222	0.263	0.081
Test/group 1	>10% NS	>10% NS	>10% NS	>10% NS
Group 4 : SR 33589B 50 mg/kg				
n = 10				
mean	**3.27**	**1.79**	**3.64**	**0.53**
SEM	0.260	0.161	0.303	0.067
Test/group 1	>10% NS	>10% NS	>10% NS	9.2% NS
Group 5 : CP 4.5 mg/kg				
n = 10	(9)	(9)	(9)	(9)
mean	**0.82**	**0.71**	**0.43**	**0.10**
SEM	0.078	0.051	0.073	0.009
Test/group 1	<0.1%***	<0.1%***	<0.1%***	<0.1%***

Table (6.4) 9 - Specific immunoglobulins day 20

MALE	IgG titer	IgG titer log(X+1)	IgM titer	IgM titer log(X+1)
Levene	<0.1%***	>10% NS	<0.1%***	>10% NS
1-ANOVA	---	<0.1%***	---	<0.1%***
Group 1 : VEHICLE				
n = 10				
mean	**1376**	**3.05**	**2048**	**3.23**
SEM	278.4	0.098	424.5	0.092
Group 2 : SR 33589B 2 mg/kg				
n = 10				
mean	**1984**	**3.20**	**1344**	**3.05**
SEM	441.9	0.101	277.3	0.087
Test/group 1	>10% NS	>10% NS	>10% NS	>10% NS
Group 3 : SR 33589B 10 mg/kg				
n = 10				
mean	**1200**	**2.96**	**2688**	**3.32**
SEM	262.6	0.121	578.8	0.110
Test/group 1	>10% NS	>10% NS	>10% NS	>10% NS
Group 4 : SR 33589B 50 mg/kg				
n = 10				
mean	**1824**	**2.78**	**1024**	**2.93**
SEM	1049.2	0.193	212.3	0.092
Test/group 1	>10% NS	>10% NS	4.5% *	>10% NS
Group 5 : CP 4.5 mg/kg				
n = 10				
mean	**55**	**1.53**	**107**	**1.90**
SEM	16.8	0.154	27.2	0.117
Test/group 1	<0.1%***	<0.1%***	<0.1%***	<0.1%***

Table (6.4) 10 - Specific immunoglobulins day 2

MALE	IgG titer	IgG titer log(X+1)	IgM titer	IgM titer log(X+1)
Levene	1.8% *	>10% NS	<0.1%***	>10% NS
1-ANOVA	---	<0.1%***	---	<0.1%***
Group 1 : VEHICLE				
n = 10				
mean	**3648**	**3.44**	**2944**	**3.41**
SEM	905.3	0.114	506.6	0.078
Group 2 : SR 33589B 2 mg/kg				
n = 10				
mean	**3136**	**3.32**	**1536**	**3.11**
SEM	951.4	0.135	289.4	0.090
Test/group 1	>10% NS	>10% NS	2.7% *	>10% NS
Group 3 : SR 33589B 10 mg/kg				
n = 10				
mean	**3584**	**3.29**	**2080**	**3.17**
SEM	1199.0	0.175	558.9	0.125
Test/group 1	>10% NS	>10% NS	>10% NS	>10% NS
Group 4 : SR 33589B 50 mg/kg				
n = 10				
mean	**3136**	**3.35**	**2368**	**3.29**
SEM	937.0	0.117	505.3	0.092
Test/group 1	>10% NS	>10% NS	>10% NS	>10% NS
Group 5 : CP 4.5 mg/kg				
n = 10				
mean	**589**	**2.68**	**112**	**1.96**
SEM	121.0	0.094	25.8	0.091
Test/group 1	0.8% **	<0.1%***	<0.1%***	<0.1%***

One mechanism that can explain all observed findings is generally considered to have a greater probability than multiple concurrent or coincident mechanisms. However, it does not seem possible to do that with dronedarone. First, some of the data patterns include changes at the LD and MD but not the HD or there are changes that do not follow a classical dose-response. Possible interpretations are:

1. the changes in numbers are fluctuations within a normal range
2. the changes are real, but not dose dependent
3. a mix up in data?

Second, most of the effects such as the cyclicity, serum and urinary electrolyte changes, changes in thyroid histopathology, some of the ECG changes, seem to fade with increased duration of exposure to the drug. Based upon the available kinetic data it does not appear that there is induction of metabolism. If anything, there seems to be some increase in plasma levels over time. Is there some receptor-based down-regulation or a specific, unidentified metabolite that decreases over time?

Looking for a one-mechanism explanation, the most obvious consideration is the thyroid-mimetic aspect of the drug's structure. Yet neither hypothyroid effects nor hyperthyroid effects fit the data. It is possible that the structure activity relationship of the drug gives mixed effects.

Hyperthyroid vs Hypothyroid effects (Goodman and Gilman 10th edition)

Hyperthyroid effects	**Hypothyroid effects**
Calorigenic effects	Skin changes, Voice changes, poor appetite
Cholesterol lowering	Hypercholesterolemia
Cardiac effects Tachycardia hypertrophy ↑ Stroke volume ↓PVR ↑ cardiac index ↑ pulse pressure angina arrhythmias	Cardiac effects Bradycardia ↓cardiac index ↑ PVR ↑ mean arterial pressure
Insulin resistance	↓insulin secretion, ↓glucose absorption in GI
Exophthalmos	Anemia
	Menstrual irregularities

2.6.6.2 Single-dose toxicity

TXA0224 Single dose oral toxicity study in mice and rats of both sexes(3/31/93 initiated). Five animals/sex/species/group were given single oral doses of 0, 1500 or 2000 mg/kg SR33589B in 10% gum Arabic followed by a 14 day observation period. There was no mortality in either sex of either species. No clinical signs were reported for mice. Rats showed piloerection, prostration and soiled urogenital areas. The 2000 mg/kg group showed ptyalism on the day of treatment. There were moderate effects on body weight gain over the course of the study.

Summary of body weight gain changes in rats and mice

Dose mg/kg	Mice % change from baseline Day1-Day15		Rats % change from baseline Day1-Day15	
	males	females	males	females
0	+41	+19	+95	+59
1500	+35	+16	+69	+44
2000	+30	+17	+70	+50

TXA0232Single dose oral toxicity study in mice and rats of both sexes (June 29, 1993 initiated). Five animals/sex/species/group were given single oral doses of 0 or 2000 mg/kg SR33589B in 0.6% methylcellulose followed by a 14 day observation period. Consistent with the previous study using a different vehicle, no deaths were seen. No clinical signs were reported for the mice. Rats again showed

ptyalism, prostration, piloerection and soiled urogenital area. Moderate body weight gain changes were seen.

Summary of body weight gain changes in rats and mice

Dose mg/kg	Mice % change from baseline Day1-Day15		Rats % change from baseline Day1-Day15	
	males	females	males	females
0	+41	+17	+113	+73
2000	+30	+19	+89	+56

TXA0231 Single dose intravenous toxicity study in mice and rats of both sexes (June 22, 1993 initiated). Five animals/sex/species/group were given single bolus intravenous doses of 0, 10, 15 or 20 mg/kg SR33589B in a vehicle of PEG400, acetic acid, buffer and glucose solution. The administration was followed by 14 days of observation. Death occurred within 5 minutes of dosing and is summarized below.

Summary of premature deaths in mice and rats after IV administration

Dose mg/kg	Mice		Rats	
	males	females	males	females
0	0	0	0	0
10	0	0	0	0
15	1	2	0	0
20	5	4	2	1
LD50	15-20 mg/kg		>20 mg/kg	

Both species showed the same clinical signs of prostration and hematuria (≥15mg/kg in both sexes of mice).

Summary of body weight gain in mice and rats after IV administration

Dose mg/kg	Mice		Rats	
	males	females	males	females
0	+31	+21	+77	+42
10	+28	+11	+76	+43
15	+26	+24	+77	+40
20	----	+23	+70	+54

TXA0290 Single-dose intravenous toxicity study in mice and rats of both sexes (April 13, 1994.) Five animals per species per sex per dose received bolus intravenous doses of 0, 10 or 15 mg/kg followed by a 14 day observation period. A vehicle of mannitol, monosidic anhydrous phosphate, glucose and water for injection. Unscheduled mortality was seen.

Summary of premature deaths in mice and rats after IV administration

Dose mg/kg	Mice		Rats	
	males	females	Males	females
0	0	0	0	0
10	0	0	0	0
15	4	3	5	5
LD50	10-15 mg/kg		10-15 mg/kg	

2.6.6.3 Repeat-dose toxicity

Study title: **Two-week oral toxicity study in the rat**

Key study findings: Target organs of toxicity were the digestive tract, reproductive tract, kidney and liver. Dyslipidosis was also seen.

One HD(160 mg/kg)m and 3 HDf were found dead from D14 to D16. Surviving HD males lost 8% of their starting bodyweight while females lost 7%. LD(30 mg/kg) and MD(70 mg/kg) groups showed the same rate of gain as the controls. The males showed dose-related increases in PCV and RBC. These changes were apparent only in HDf. Both absolute and percentage of reticulocytes were decreased in the HD groups. Lymphocytes, monocytes and neutrophils were increased in the HD groups of both sexes. Serum urea was increased in the drug-treated groups of both sexes. Serum glucose was increased in the MD and HD groups. Albumin was decreased in the HD of both sexes. ALT and AST were increased in the HD groups of both sexes. Serum electrolytes were altered in both sexes with dose-related increases in sodium and potassium (≥30 mg/kg) with decreased calcium at the HD. There were slight increases in renal weight in the HD of both sexes. Significant increases were seen in adrenal, lung, eye and brain weight. In females, uterine weight showed a dose-related decrease. In males, prostate weight showed a dose-related decrease. Drug-treated females showed a predominance of being paused in diestrus. HD males showed testicular changes such as debris, retained spermatids and multinucleated cells. Atrophy of the seminal vesicles was also noted. In the digestive tract, gross changes (≥70 mg/kg) included inflammatory and/or ulcerative lesions in the pancreatic ducts, liver necrosis, erosive/ulcerative lesions in the glandular stomach. Other histologic changes of note included 2 HD m and 2 HD f with necrotic foci in the liver. Other HD changes included fibrous changes in the biliary tract, biliary hyperplasia. Spleen, thymus, lung and mesenteric lymph node primarily at the HD were reported to show infiltration with foamy macrophages. Electron microscopy indicated that dyslipidosis would be an appropriate term for the vacuolation of the Kupffer cells studied. Chronic lymphoid atrophy of the spleen was also reported for the HD (m+f) as was epithelial hyperplasia and hyperkeratosis of the esophagus and stomach. While some changes are probably stress related (splenic lymphoid atrophy, thymic involution, adrenal hyperplasia), other changes may be beyond that, possibly due to weight loss, such as fatty atrophy of the bone marrow and the degenerative change in the male reproductive tract.

Study no.: TXA879
Conducting laboratory and location: Sanofi, Montpelier Cedex, France
Date of study initiation: April 7, 1993
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589B batch 92.01 in 0.6% methylcellulose.
Doses of 0, 30, 70 and 160 mg/kg/day were given by oral gavage to groups of 10/sex/group. The doses were based on a preliminary 8-day study that used doses of 100, 250 and 600 mg/kg/day

Methods: Test material was given daily for 16-20 days with the last dose given 24 hours before euthanasia and necropsy.
ECGs: days -5, +8 4 rats/sex/group
Ophthalmoscopy: days -6, +10 5/sex/group
Bodyweight: pre treatment then weekly
Food consumption: weekly
Hematology: days -13, +13
Clinical chemistry: days -9, +15
Urinalysis: overnight collection following administration of 10ml/kg tap water 5/sex/group,
Days -1 and +10
TK: 1,2,4,8 and 24 hours post-treatment, the last treatment was administered within 24 hours before necropsy/
Necropsy: D16-D21
Liver samples : 5/sex/group used for assessment of CYP450 effects (reviewed under ADME section).
Statistical significance: * $1.0\% < p \leq 5.0\%$; ** $0.1\% < p \leq 1.0\%$; *** $p \leq 0.1\%$

Results

Unscheduled mortality: 3 HDf, 1HDm. Prior to death, the animals were thin, with dirty urogenital areas. One female was reported to show prostration and coma. Later findings were decreased thymus volume, white hepatic foci, thinning of the gastric wall with irregular mucosa and erosions. The mesenteric lymph nodes were enlarged with yellowish discoloration. Changes in the stomach and lymph nodes were seen in the LD and MD groups also. Clinical signs reported for the HD group were:
Ptyalism: all animals week 2
Health deterioration all animals week 2 (soiled muzzle, ↓activity, piloerection, soiled urogenital area (5/10males, 6/10 females), thinness, hypothermia)
Hematuria reported for 1/10 males.

Both HD males and females lost weight over the course of the study.

Summary of body weight gain

Dose	Males			Females		
Mg/kg	Day 1	Day 8	Day 14	Day 1	Day 8	Day 14
0	279±8.4	336±9.0	367±12.7	214±3.6	237±5.0	243±4.4
30	273±6.5	331±7.5	354±9.0	218±3.5	254±4.5*	262±4.9*
70	271±5.7	331±6.7	354±7.8	229±3.0**	262±4.6***	270±4.8***
160	277±4.7	283±7.7***	256±12.4***	223±2.7	214±2.4**	208±5.7***

***<0.1% by ANOVA

Hematology in males showed a dose-related increase in RBC and PCV. Hemoglobin was increased at the HD group. Absolute and percentage of reticulocytes were significantly decreased.

Female data showed increased RBC, hemoglobin and PCV in the HD only. Absolute and percent reticulocytes were also decreased.
Both sexes showed significantly increased neutrophil, monocyte and lymphocyte absolute percents at the HD.

Hematology summary

parameter	Males				Females			
	0	30	70	160	0	30	70	160
Rbc $x10^3$	7873	8007	8076	8821	8202	7832	8002	8895
Hb	151	157	153	162***	157	155	144*	162
Pcv	46	48	48	50***	47	46	44	50
Retics%	2.8	3.2	2.8	1.0	2.0	2.7	2.4	0.9
Reticx10^3	215	250	228	834	166	209	191	77

Absolute and normalized bone marrow cellularity was also decreased (NS) at the HD. However, a dose-response was not apparent.

Heart rate showed dose-related decreases in both sexes. T-wave amplitude was decreased primarily at the HD of both sexes.

Summary of ECG findings

Dose mg/kg	males		Females	
	hr	T wave	Hr	T wave
0	402.7±34.17	0.257±0.013	422.3±30.46	0.256±0.026
30	402.5±21.11	0.215±0.019	417.7±9.95	0.208±0.036
70	384.3±17.22	0.248±0.013	363.2±49.11	0.175±0.024
160	283.0±21.20*	0.178±0.019*	303.1±34.40	0.188±0.013

Hematology and Clinical Chemistry

Monocytes and neutrophils were increased in the HD males and to a lesser extent in the HD females. The results are suggestive of a subacute process.

Differential Summary

Dose	Males			Females		
mg/kg	lymphocytes	monocytes	Neutrophils	lymphocytes	monocytes	Neutrophils
0	9348±877	166±50	1353±188	6066±392	157±30	825±103
160	10643±1197	435±122***	3624±851***	7436±961**	200±45	4044±690***

***p<0.1%

Serum urea and glucose showed increases at the HD of both sexes. Albumin and triglycerides were decreased at the HD of both sexes. Cholesterol showed a slight decrease in HDf and a very slight increase in males. AST and ALT were increased in HD groups of both sexes.

Summary of Clinical Chemistry findings

Parameter	Males: dose in mg/kg			
	0	30	70	160
Urea (mM)	2.93±0.20	3.17±0.15	3.15±0.21	6.97±0.94**
Glucose (mM)	5.93±0.20	5.66±0.14	5.88±0.17	6.71±0.27*
Albumin g/l	32.1±0.23	33.1±0.46	33.3±0.37	30.8±0.68
Triglycerides(mM)	1.05±0.16	0.78±0.05	0.82±0.02	0.42±0.05**
Cholesterol (mM)	2.14±0.13	2.38±0.08	2.38±0.07	2.33±0.17
ALT (IU/l)	22±1.9	21±0.7	24±1.7	67±13.8**
AST (IU/l)	53±2.8	57±2.6	58±3.9	94±16.8*
Females				
Urea (mM)	4.37±0.31	4.28±0.25	4.05±0.22	7.94±1.45*
Glucose (mM)	5.97±0.17	5.85±0.12	6.07±0.12	6.31±0.58
Albumin g/l	36.4±0.37	34.5±0.48	33.5±0.45	30.8±0.62***
Triglycerides(mM)	0.061±0.05	0.67±0.03	0.82±0.05*	0.39±0.07
Cholesterol (mM)	2.87±0.12	2.78±0.15	2.84±0.13	1.98±0.29
ALT (IU/l)	19±1.2	20±1.4	22±1.2	91±23.9
AST (IU/l)	55±2.7	52±1.1	55±3.3	115±32.9

*p<3% **p<0.2% ***p<0.1

Serum sodium increased in both sexes while serum calcium decreased.

Summary of serum electrolyte changes

	Males: doses (mg/kg)			
	0	30	70	160
Na mM	145.9±0.35	148.0±0.47	147.8±0.44	149.8±0.51***
K mM	4.68±0.10	4.89±0.17	4.84±0.08	4.90±0.07
Ca mM	2.89±0.02	2.89±0.04	2.88±0.03	2.59±0.04***
	Females			
Na mM	145.6±0.43	146.2±0.42	147.0±0.37*	151.3±0.31***
K mM	4.62±0.08	4.78±0.12	5.05±0.16	5.14±0.13*
Ca mM	2.89±0.03	2.87±0.03	2.84±0.02	2.58±0.07**
*p<1.5%, **p<0.6%, ***0.1%				

Organ Weight Summary

In males, liver weight was slightly decreased at the HD. Thymic weight showed a dose-related decrease. Weights of the eyes, brain, lungs, spleen, pituitary and testes showed increases. No explanation as to these changes was given.

Summary of relative organ weight changes: males

	Males: dose (mg/kg)			
	0	30	70	160
Liver g/kg	41.59±1.4			35.18±1.1**
Spleen g/kg	2.51±0.14			2.76±0.15
Kidneys g/kg	7.18±0.23			8.56±0.61
Adrenals mg/kg	158±6.4	172±9.3	165±7.2	330±23***
Thymus mg/kg	1945±113	1767±63	1729±131	910±136***
Lungs g/kg	4.05±0.11	4.25±0.15	4.21±0.12	5.15±0.30**
Eyes g/kg	0.778±0.023	0.845±0.029	0.803±0.04	1.141±0.07***
Brain g/kg	5.96±0.17			8.08±0.41***
Pituitary mg/kg	42.2±2.45			50.6±4.91
Testes g/kg	10.4±0.32			14.0±0.64
Prostate g/kg	1.67±0.06	1.59±0.14	1.43±0.10	1.20±0.16

In females, spleen, adrenal, lung, brain, eye, pituitary and kidney weight increased. Uterine weight decreased.

Summary of relative organ weight changes: females

Liver g/kg	40.76±1.00			40.41±0.87
Spleen g/kg	2.83±0.19	3.01±0.14	3.22±0.13	3.49±0.22
Kidneys g/kg	7.29±0.14			8.39±0.36**
Adrenals mg/kg	313±10			475±23***
Thymus mg/kg	2132±134	2054±129	1958±118	868±144***
Lungs g/kg	5.13±0.15			5.85±0.19*
Eyes g/kg	1.149±0.05			1.443±0.082***
Brain g/kg	8.19±0.16			9.78±0.20***
Pituitary mg/kg	66.2±2.54			74.2±4.31
Uterus g/kg	2.46±0.24	2.26±0.24	1.97±0.15	1.49±0.12*
Ovaries mg/kg	440±30	416±19	449±18	451±34

*p<1.2%, **p<0.2%, ***p<0.1

Histological findings

Foamy macrophages were reported for the spleen, thymus and lung, primarily at the HD. Drug-treated females were paused in diestrus with no NOAEL identified. Testicular degeneration was reported in HD males.

Summary of histological findings

	Dose mg/kg							
	0	0	30	30	70	70	160	160
	m	f	m	f	m	f	m	f
Mammary hyperplasia	6		3	1	2			
Liver: necrotic foci						1	2	2
Bile duct: fibrous foci							3	3
Biliary hyperplasia							1	3
Spleen chronic lymphoid atrophy					1		6	6
Spleen: infiltration w/foamy macrophages							3	7
Thymus: infiltration w/foamy macrophages							7	8
Lung: infiltration w/foamy macrophages	4	3	4	2	2	6	9	9
Mesenteric lymph node: infiltration w/ foamy macrophages	0	0	3	1	3	4	10	10
Endometrial hyperplasia		8		6		9		1
Estrus cycle phase:								
Diestrus		1		5		4		8
Proestrus		2		2		0		0
Estrus		0		0		1		0
Metestrus		7		3		5		0
Muciparous aspect of vaginal epithelium		0		2		2		6
Male genital tract								
Vacuoles in seminiferous tubule epithelium	0		0		0		6	
Decreased # of sperm in epididymides	0		0		0		1	
Debris,spermatids, multinucleated cells in	0		0		0		6	

Seminiferous tubule lumens or epydid. Atrophy of seminal vesicles	0		0		0		3	
Epithelial hyperplasia/hyperkeratosis of esophagus							10	10
Epithelial hyperplasia/hyperkeratosis of stomach							6	4

Study title: Three -month oral toxicity study in the rat

Key study findings: There were no consistent body weight effects. HR was decreased at doses ≥5 mg/kg (both sexes). PR interval was increased at 60 mg/kg (HD) in both sexes. There were no clear effects on lymphocyte subpopulations. There was a mild increase in IgM in HDm and a dose-related increase in IgM in females. T3 was decreased slightly in HDm and significantly decreased in all groups of drug-treated females but with no dose response. Prolactin levels do not show a consistent pattern.The significance of the hormone results is not clear. Clinical chemistry changes were consistent with the previous study. There were changes in triglycerides and cholesterol seen at day 28 that did not persist to day 100. Histologic changes were also consistent with previous studies and included foamy macrophage infiltration, synchronization of the drug-treated females in diestrus and splenic congestion. Thyroid hyperplasia was manifested as columnar follicular epithelium, an effect that was more apparent in males (≥5 mg/kg). Infiltration of several organs by foamy macrophages was reported ≥5 mg/kg for lungs, tracheobronchial lymph nodes, popliteal lymph nodes, mesenteric lymph nodes and ≥17.5 mg/kg for the ileum and the femoral bone marrow. Heart weight was increased 5-11% in females and 4-9% in males.

Study no.: TXC0887
Conducting laboratory and location: Sanofi, Montpellier Cedex, France
Date of study initiation: May 13, 1993
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589B batch 92.01 purity in 0.6% methylcellulose
Doses of 0, 5, 17.5 and 60 mg/kg/day were used

Methods
Groups consisted of 15/sex/dose for the main study and 5/sex/dose for the tk study.
The main study animals were dosed daily for 90 days.
The toxicokinetic animals were treated for 28 days
Observations:
Clinical signs
ECG: Days -2, +97; 2-4 hours after dosing on 5 conscious rats /sex/dose from main study
Ophthalmoscopy: Days -3 and Day +90 on last 10 animals/sex/main study groups
Hematology: Days +26 and +96
Lymphocytes: Day 96. MAb used for determination of CD4, CD8 and B lymphocytes by FAC analysis

IgG and IgM: radial immunodiffusion assays on blood collected at time of necropsy
Clinical chemistry: days 28 and 100
Hormone levels: T4, T3, TSH, prolactin
Urinalysis: 5/sex/main group on overnight urine samples after water loading
Partly done by reagent strip
Ion and creatinine excretion: day 92
TK: blood collected 1,2,4,6 and 24 hours after last treatment on day 28 from the tk groups and at terminal necropsy from 2 rats/sex from the main study groups.
Necropsy: day 103-110 for surviving main group animals

Statistical analysis: p values included in the tables are associated with the following statistical significance:

*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

One MD was found dead, most likely due to a dosing accident.
The males started the study with body weight differences between the groups.

Summary of body weight effects

	Males: dose groups (mg/kg/day)			
	0	5	17.5	60
Day 1	253±2.7	241±2.6	251±3.6	253±3.4
Day 106	539±13	462±21	525±28	486±24
Females				
Day 1	207±3	209±4	206±3	201±3
Day 106	314±12	332±12	351±15	317±4

Hematology

Both sexes showed slight decreases in RBC, Hb and PCV. The significance, if any, is questionable.

At day 96, there was slight difference in the lymphocyte subpopulations. The significance, if any, is not clear.

Summary of lymphocyte populations: mean %±SEM and (mean absolute ($10^3/mm^3$))

	Males: dose (mg/kg/day)			
	0	5	17.5	60
CD4+	44.7±5.5	52.8±4.2	51.7±4.9	52.9±3.7
CD8+	3.5±0.9 (0.26)	4.6±0.9 (0.28)	6.3±1.1 (0.39)	6.2±1.0 (0.42)
B	27.0±2.8(2.07)	19.8±1.3(1.21)	18.7±1.2*(1.19)	23.5±1.5 (1.54)
Females (mg/kg/day)				
CD4+	62.6±1.9	62±2.7	62.8±2.8	61.3±3.5
CD8+	5.5±0.8	6.8±1.1	7.4±1.1	7.6±1.54
B	18.9±0.7	18.7±1.5	16.3±0.7	19.4±2.6

No changes of apparent significance in bone marrow cellularity.

Electrocardiography

ECG changes of note are summarized below.

Summary of ECG changes (mean±SEM)

	Males: doses (mg/kg/day)			
	0	5	17.5	60
HR	485±5	396±42	363±17*	350±19*
P R(0.01s)	4.8±0.09	5.0±0.34	5.1±0.16	5.3±0.17
Females				
HR	489±17	358±31	385±30	326±45**
P R(0.01s)	4.6±0.13	4.8±0.24	4.7±0.45	6.1±0.52**

Clinical chemistry

The changes in urea, creatinine, cholesterol and triglycerides were consistent with those seen in the previous study.

> Males: day 28: urea↓ with no DR relationship, creatinine ↑ very slightly at MD and HD , alk phos ↓ HD, triglycerides ↓, dose related increase in cholesterol.
>
> Males: day 100: dose related increase in urea, creatinine increased at MD + HD, glucose significantly increased at MD, triglycerides and cholesterol were decreased
>
> Females: day 28: dose related increase in glucose and creatinine, urea decreased (no DR), alk phos slightly increased at MD and HD.
>
> Females: day 100: glucose significantly increased at MD and HD, creatinine showed a dose-related increase, urea was inconsistently decreased, cholesterol was slightly decreased.

Total protein, albumin and globulins: No dose-related or consistent patterns were apparent.

In both sexes, the electrolytes measured were inconsistently affected at day 28 and the changes were of questionable significance, if any. By day 100, serum K+ was increased in the drug-treated groups without a dose-response relationship.

Summary of Day 100 serum electrolyte changes

	Males: dose (mg/kg/day)			
	0	5	17.5	60
Na mM	147.7±0.56	148.9±0.31	149.6±0.37	148.9±0.42
K mM	4.83±0.10	5.20±0.13	5.24±0.12	5.11±0.10
Cl mM	100.2±0.57	103.4±0.29***	104.1±0.58***	104.2±0.49***
	Females: Dose (mg/kg/day)			
Na mM	148.4±0.38	148.2±0.46	148.8±0.30	147.7±0.41
K mM	4.64±0.10	4.61±0.10	5.13±0.17*	5.03±0.09**
Cl mM	103.1±0.46	104.6±0.32*	105.3±0.37***	105.3±0.51**

IgM was increased at the HD for males and in females showed a dose-related increase.

Summary of Immunoglobulin findings day 103

	Males: dose (mg/kg/day)			
	0	5	60	160
IgM mg/l	1170±52	1162±34	1166±39	1319±49
	Females			
IgM mg/l	1150±29	1191±49	1268±56	1500±35***

***p<0.1%

Endocrine assessment

There was a mild, dose-related increase in TSH and inconsistent changes in T3 and T4.

Summary of thyroid tests, day 98

	Male: dose mg/kg/day			
	0	5	17.5	60
TSH ng/ml	9.6±0.89	10.1±1.1	10.3±0.92	11.3±1.7
T3 nM	1.02±0.06	0.81±0.09	0.97±0.04	0.78±0.05
T4 nM	34.34±1.79	31.03±1.68	35.72±0.83	31.93±1.54
	Female			
TSH ng/ml	6.7±0.43	5.9±0.20	6.9±0.44	8.4±0.56
T3 nM	1.43±0.05	0.91±0.03***	0.96±0.04***	0.99±0.04***
T4 nM	35.66±2.47	34.15±1.07	31.89±1.37	34.52±1.17

***p<0.1%

There was no effect on male prolactin levels. In females, there were non-significant decreases in prolactin at the LD and MD and a non-significant increase at the HD. What do the results mean? Typically, prolactin levels are increased only on the day of proestrus

(Haschek and Rousseaux, Handbook of Toxicologic Pathology, edition I. p.930). Inclusion of estrogen and progesterone levels as well might have been more informative.

Prolactin levels day 98

	Males: dose mg/kg			
	0	5	17.5	60
Prl ng/ml	19.5±5.8	25.1±5.9	23.3±6.6	17.1±7.6
	Females			
Prl ng/ml	17.6±7.42	4.8±1.03	2.1±0.30	35.6±28.46

Urinalysis

Males showed greater excretion of Na (MD and HD), K(HD) and Cl(HD). Females showed inconsistent changes, with decreased Na excretion at the HD. None of the changes were statistically significant.

Organ weight changes

Absolute organ weight changes: spleen was increased at the HD in both sexes; thymus in both sexes showed dose-related decrease; heart was increased in females; prostate was inconsistently decreased; uterus was decreased at the HD; ovaries were increased at MD, HD.

Normalized organ weight changes: Increased spleen at the HD in both sexes ($p<0.001$), decreased thymus in both sexes, heart weight increased in both sexes, decreased uterus at HD, increased ovary weight(HD). Red blood cell congestion of the spleen was noted as a finding in the HD groups, probably accounting for the increased organ weight.

Organ weight changes are summarized below.

Summary of normalized organ weight changes

	Males : dose (mg/kg)			
	0	5	17.5	60
spleen	1.742±0.05	1.847±0.06	1.794±0.08	2.149±0.08***
Thymus	723±33.4	656±31.3	598±31.5*	600±26.3*
Heart	2.89±0.07	3.20±0.06** (11)	3.03±0.05** (5)	3.15±0.065* (9)
	Females			
Spleen	2.152±0.09	2.241±0.11	2.201±0.06	2.852±0.14***
Thymus	1029±46.4	915±66.2	868±75.1	827±40.4
Heart	3.42±0.08	3.55±0.07 (4)	3.74±0.10*(9)	3.72±0.09 (9)
uterus	2.252±0.17	2.12±0.17	2.397±0.24	1.84 (-18)
ovary	298±19.5	289±16.5	303±11.1	334±12.5 (12)

*<1.3%, **0.3%, ***<0.1% Numbers in parentheses ( ) are percent difference from control value.

Histologic Findings

The histologic changes were consistent with previous studies and included foamy macrophage infiltration, synchronization of the females in diestrus, and a congested spleen. Thyroid changes were also apparent in columnar follicular epithelium, a change associated with hyperplasia of the organ.

Summary of histological findings

	Females: dose (mg/kg)							
	0		5		17.5		60	
Cycle: diestrus	4		10		8		10	
Proestrus	1		1		2		1	
Estrus	1		2		3		1	
Metestrus	9		2		2		3	
	m	f	m	f	m	f	m	f
Thyroid : columnar follicular epithelium	9	1	15	0	14	2	15	7

Mesenteric lymph nodes from 3 HD animals were examined by electron microscopy to determine the macrophage morphology. The heterogenous cytoplasmic inclusions were deemed to be probably of lipid nature.

Summary of microscopic infiltration by foamy macrophages

	Dose (mg/kg)							
	0		5		17.5		60	
	m	f	m	f	m	f	m	f
Lungs	6	5	5	5	5	4	7	11
Tracheobronchial lymph nodes	-	-	1	3	1	2	2	2
Popliteal lymph nodes	-	-	5	2	6	5	8	3
Mesenteric lymph nodes	-	-	4	3	7	7	9	11
Ileum	-	-	-	-	1	0	1	1
Femoral bone marrow	-	-	-	-	-	1	1	-

The TK data was reviewed in the ADME section. However, as a reminder, there was accumulation of material from day 30 to day 90. The sponsor calculated an accumulation factor, R. This is summarized in the table:

$R=AUC_{day90}/AUC_{day\ 30}$

Sex	Dose (mg/kg/day)	SR33589	SR35021
M	17.5	2.1	-
F	17.5	1.7	-
M	60	2.5	1.8
F	60	1.9	3.2

Study title: Six-month oral toxicity study in the rat

Key study findings: As seen in the 3-month study changes in renal function were demonstrated by slightly increased creatinine levels in females (≥10 mg/kg after 6 months) and in males and females at 50 mg/kg (after 3 and 6 months). Serum chloride levels were increased in both sexes at all doses. There were no histologic changes reported in association with the renal findings. Foam cell infiltration was seen primarily in lungs at 50 mg/kg (HD), in some lymph nodes of animals of both sexes at the end of treatment and only in males at the end of the recovery phase. Foam cell infiltration was associated with an increase in perivascular lymphoid hyperplasia. There was an alteration in thyroid function in males manifested by a slight decrease in T3 levels and increased incidence of thyroid columnar follicular epithelium. Heart rate was decreased in males after 3 months and in females after 3 and 6 months of treatment. Increased QTa was also seen. Adding up the individual animal results does not always give me the same numbers as the sponsors have in their summary tables.

Study no.: TXC0986
Conducting laboratory and location: Sanofi, Montpellier Cedex, France
Date of study initiation: December 5, 1995
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589B batch 5 SNP505 purity in 0.6% methylcellulose

Methods
Rats received daily oral gavage doses of 0, 2,10 and 50 mg/kg/day for 191-198 days for the main study or for 30 days for the toxicokinetic study. The reviewer's summary of group numbers is shown below:

Summary of numbers of animals assigned to groups

Dose group(s)	Number of rats
Control , 50 mg/kg/day	30 /sex/group
2 mg/kg/day and 10 mg/kg/day	20/sex/group
Satellite groups for TK study	5/sex/group

Reversibility: 6 weeks (48-49 days) for 10 animals/sex in the control and HD groups

Doses were based on TCX887 where the LD of 5 mg/kg/day caused slight dyslipidosis, some hormonal changes, occasional increases in AST activity with no histologic changes. The HD of 60 mg/kg caused the same changes to a greater degree and in addition, some digestive effects, renal function changes and splenic congestion.

Main Study
ECG: days -4, 98 and 186, 2-4 hours post dose for 5rats/sex/group; day 242 on the other 10 animals/group
Ophthalmoscopy: day-1, 86, 177 on 10/sex/group from main study
Bodyweight: pretreatment then weekly
Food intake: weekly

Hematology: day -12, 91, 182 males // day -11, 92, 183 for females//day 238 in both sexes in recovery groups
Lymphocyte subpopulation: day 185 from 5 rats/sex/group (not the animals used for hormone level detection) CD4 and CD8 cells by FAC analysis
Clinical Chemistry: Day -8, 94, 189 males//day -7, 95, 190 females//day 241 recovery grp
Hormones: T4, T3, TSH, prolactin measured on day 185 and from the recovery animals day 245
IgG and IgM: radial immunodiffusion assay used on blood collected at necropsy
Urinalysis: day -4, 88, 179 overnight collection 5/sex/group after water loading
TK: satellite groups: day 30: 1,2,4,6,24 hours 1/sex/group/time
Main groups: day 191-196: 1,2,4,6,24 hours 2/sex/group/time at necropsy
Necropsy: D191-199. Gross examination, organ weights, collection of tissues for light Microscopy, liver evaluation by EM

Statistical analysis: p values included in the tables are associated with the following statistical significance:
*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Reversibility Phase
ECG day 242 5/sex/group
Bodyweight and food: weekly
Hematology: Day 238
Clinical chemistry: Day 241
Hormones: Day 245
Necropsies: Day246- D247

Clinical signs seen at the HD include: ptyalism in most animals seen a few minutes after administration, sometimes seen after 71 days of dosing; soiled urogenital area in 6 females; waste of food in 2 males and 10 females.
HD males gained somewhat less than the other groups during the last 80 days of dosing but appeared to regain ground during the recovery phase. The HD females gained more weight than the other dose groups.

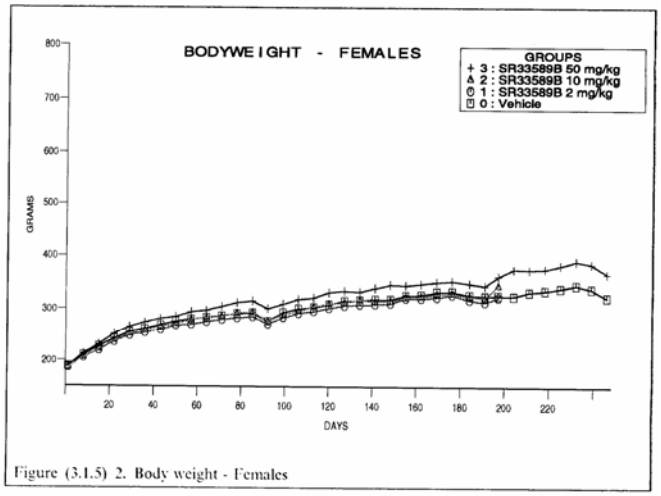


Figure (3.1.5) 2. Body weight - Females

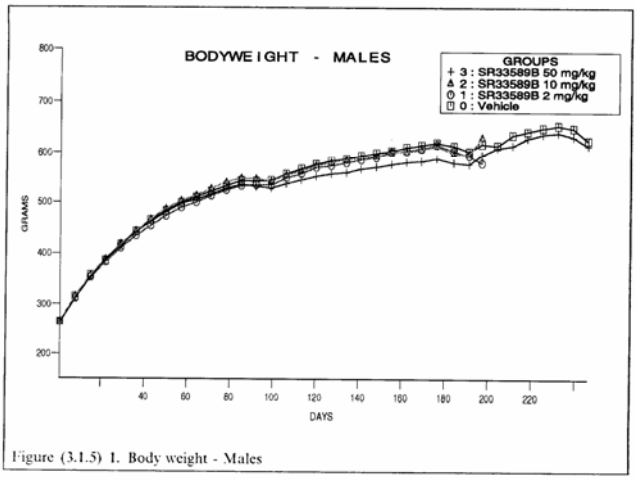


Figure (3.1.5) 1. Body weight - Males

It should be noted that HD females showed markedly increased food consumption for the duration of the study and recovery period.

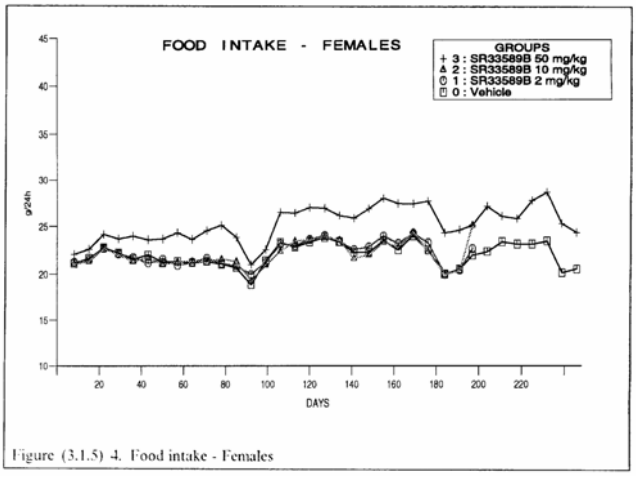


Figure (3.1.5) 4. Food intake - Females

ECG

In males, heart rate was inconsistently decreased in the LD and MD groups. HR changes in the HD group persisted into the recovery period. In females, HR was decreased only at the HD. In the recovery period, HR increased to above control levels. PR interval was increased day 98 in males only. QTc showed an increase only on day 186. T wave

amplitude was markedly increased in females during the recovery period: control 0.0176±0.03 vs HD 0.299±0.015 p<0.02). Some of the ECG changes are summarized on the next page.

Summary of ECG changes

	Males: Dose mg/kg			
	0	2	10	50
HR bpm Day 98 Day 186 Day 242	 472.5±7 407.5±28 406.2±38	 444.7±20 385.4	 462.2±13 412.5±18	 409.0±7 371.3±22 370.4±12
QTa (0.01s) day 186	3.7±0.15	3.8±0.12	4.3±0.44	4.3±0.17
QTc day 186	3.03±0.185	3.03±0.15	3.51±0.356	3.38±0.16
	Females			
HR bpm Day 98 Day 186 Day 242	 483.6±12 417.3±18 351.5±23	 482.6±6 427.8±26	 493.1±7 451.7±12	 395.8±17** 322.2±13** 415.7±26
QTa (0.01s) day 186	3.6±0.08	4.2±0.16**	4.1±0.12*	4.1±0.25
QTc day 186 Day 242	3.02±0.06 2.85±0.20	3.51±0.12**	3.55±0.15*	2.98±0.19 3.33±0.21

*<1.4%, **<0.8%

Hematology and Clinical Chemistry

There was a slight but significant decrease (p<0.01) in hemoglobin (156 ± 1.1 vs control value of 161±0.9) in HD males only seen by day 182. There was a slight increase in the HD group by the end of the recovery (158±1.1).
Triglycerides increased in HD females: 0.64±0.040mM vs 0.50±0.020mM Control, (p<0.03).
IgG and IgM showed slight increases in HD males.

There was no apparent effect on TSH and only a minimal effect on T3 levels in males. T3 in females was increased over control levels in the LD and MD groups.

Summary of Thyroid hormone changes

Dose mg/kg	T3 (nM)		T3 (nM)	
	M Day 185	M day 245	F Day 185	F Day 245
0	0.98±0.04	0.72±0.05	0.82±0.04	0.96±0.06
2	0.97±0.04		1.24±0.06***	
10	0.91±0.06		1.12±0.05***	
50	0.80±0.04*	0.62±0.035	0.82±0.06	0.66±0.05**

*<1.8%, **0.2%, ***0.1%

Prolactin levels were decreased in the drug-treated females during the treatment period and increased over control in the recovery period.

Summary of prolactin values (ng/ml)

Dose mg/kg				
	M Day 185	M day 245	F Day 185	F Day 245
0	33.4±7.8	38.9±8.9	68.9±27.5	141.5±25.1
2	36.6±6.3		32.5±6.6	
10	55.8±6.3		27.3±9.5	
50	22.5±5.8	25.0±3.5	26.4±6.4	294.8±77.8

There were no apparent findings of significance in either the lymphocyte subpopulation analysis or the bone marrow cellularity. IgG and IgM were increased in the HD males. IgM was increased in the HD females.

	IgG mg/l		IgM mg/l	
	males	females	Males	females
vehicle	10242±593	11027±645	1199±47	1213±41
10 mg/kg	10211±899	10557±1159	1296±44	1253±41
30 mg/kg	13318±1150*	10196±855	1390±41*	1474±44***

*1.0%<P<5.0%, ***P≤0.01%

Urinalysis: no findings of apparent biological significance.

Organ weights

There was a slight increase in spleen weight in HD females. Difference persisted at the end of the recovery phase. Relative lung weight was minimally increased in the MD and HD males at the end of the main study. No difference was apparent at the end of the recovery period. There were no obvious differences in pituitary weight at day 191. At the end of the recovery period both sexes showed increased pituitary weight.

Relative organ weights

	Females Dose: mg/kg			
	0	2	10	50
Spleen day 191 Day 246	1.985±0.05 2.050±0.8	2.063±0.08	1.938±0.07	2.284±0.09* 2.139±0.05
Lungs day 191	4.14±0.11			4.36±0.16
Pituitary Day 246	53.8±5.37			60.2±5.41
Ovary day 191 Day 246	324±14.7 346±38.2	312±12.2	345±12.1	347±16.4 265±39.1
Uterus day 191 Day 246	2.474±0.16 2.322±0.21	2.686±0.20	2.226±0.21	1.973±0.12 2.25±0.11
	Males			

Spleen day 191 day 246	1.503±0.05 1.558±0.09	1.614±0.04	1.609±0.07	1.721±0.06 1.643±0.15
Lungs day 191	3.14±0.079		3.22±0.09	3.32±0.108
Heart day 191	2.86±0.07	2.99±0.07	2.92±0.06	3.14±0.10
Pituitary day 246	19.6±1.06			25.4±1.32**

Histological findings

Macrophage infiltration was noted for several lymph nodes, but without the designation of "foam filled". There were no apparent differences for the incidence of the finding between treated and control animals.

summary of histological findings

	Dose mg/kg							
	0		2		10		50	
	m	f	M	f	m	f	m	f
Lungs: perivascular lymphoid hyperplasia	4	6	1	1	3	0	15	12
Lungs: foam cell infiltrate	1	2	1	0	0	1	5	3
Lungs: foam cell foci	8	10	3	1	3	2	16	13
Tracheobronchial lymph nodes: macrophage infiltrate	3	6	2	5	1	6	8	4
Thyroid : follicles with columnar epithelium	10	7	8	4	6	3	18	9

Toxicokinetic Parameters

The female rats showed greater plasma levels of parent drug and metabolite than the males. The increases in AUC were greater than proportional.

Dose mg/kg	SR33589 Cmax mg/l		SR33589 AUC_{0-24} mg.h/l	
	male	female	Male	female
	Day 30			
2	0.022	0.000	Nc	Nc
10	0.105	0.093	0.173	0.498
50	0.281	0.339	1.671	3.057
	Day 191			
2	Nd	0.012	Nc	Nc
10	0.110	0.077	0.366	0.369
50	0.527	0.537	4.078	6.331
	SR35021 Cmax mg/l		SR35021 AUC_{0-24} mg.h/l	
	Day 30			
2	0.000	0.000	Nc	Nc
10	0.000	0.000	Nc	Nc
50	0.047	0.073	0.158	0.213
	Day 191			
2	0.000	0.000	Nc	Nc
10	0.000	0.000	Nc	Nc
50	0.168	0.154	1.173	2.278

Study title: 4-day intravenous dose-range finding study in the rat

Key study findings: Very poor local tolerability was demonstrated by marked to severe tail edema and necrosis at all doses of drug. No NOAEL was identified in this study for decreased PCV and Hb, increased serum glucose and serum cholesterol. At doses ≥6 mg/kg (MD), ALT and AST were increased. Adhesions and yellowish areas were seen grossly at 12 mg/kg. Albumin was decreased in females at all doses and was decreased in males only at the HD. Red urine was reported for ≥6 mg/kg. There was a slight increase in normalized spleen weight in both sexes, that was dose-related in males. The sponsor identified the target organ of toxicity as the liver.

Study no.:DDO0499
Conducting laboratory and location: Sanofi Recherche, Montpelier Cedex, France
Date of study initiation: January 25,1993
GLP compliance: statement included
QA report: yes ( ) no ( )
Drug, lot #, and % purity: SR33589 batch RKJ21, purity
Vehicle: PEG400, acetic acid/sodium acetate buffer

Methods: Sprague-Dawley rats (CD(SD)BR), 3/sex/group were given intravenous doses of 0,3,6 or 12 mg/kg/day of drug for 4 days. Drug was given via the tail vein.
Observations: Daily clinical examinations, bodyweight (day1 and 3), food intake (day3), Hematology(day 4), clinical chemistry (day 4), necropsy (day 5)

Statistical analysis: p values included in the tables are associated with the following statistical significance:

*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Results

Clinical Signs:

Clinical signs reported were

6 mg/kg: Red urine on day 3 in 2f/3
12 mg/kg: prostration on Day 1 (1 m/3), “decubitus” lasting several minutes after treatment on day 1 and day 2 in 6/6 HD animals.

The tail showed severe dark discoloration, marked to severe tail edema and necrosis from day 2 (≥3 mg/kg)

Hematology and Clinical Chemistry

PCV and absolute number of reticulocytes decreased in the drug-treated males. While PCV decreased also in the females, there was no apparent change in absolute reticulocyte number. Hemoglobin concentration also decreased.

Summary of hematology changes

Dose mg/kg	PCV	Reticulocytes/mm3 (%)	Hb g/l
	males		
0	48±2.1	674947±24160 (9.3)	138±4.7
3	44±1.0	730514±12936 (10.9)	133±1.5
6	42±1.5	571873±38310 (9.0)	122±8.2
12	40±2.6	565679±46712 (8.5)	126±8.4
	Females		
0	43±1.5	294952±25117 (4.4)	130±2.0
3	38±2.3	478948±66851 (7.4)	126±9.6
6	39±0.7	385447±51880 (6.4)	114±6.1
12	34±1.0	335101±67471 (6.0)	102±1.5

Summary of neutrophils and lymphocytes

Dose	Neutrophils %(absoloute/mm3)		Lymphocytes % (absolute/mm3)	
mg/kg	males	females	males	females
0	12±1.8 (1274±259)	7±4.0 (587±458)	86±2.5 (9404±1350)	87±6.0 (5847±1848)
3	11±5.5 (958±403)	18±4.5 (1447±291)	85±7.5 (8379±1834)	78±2.8 (6299±827)
6	23±5.7 (2627±761)	20±3.1 (1887±83)	71±6.2 (7929±472)	74±1.7 (7237±896)
12	29±8.5 (3509±1291)	19±7.0 (1538±854)	68±9.3 (7622±442)	78±5.0 (5724±993)

Neutrophils (both percentage and absolute) increased in both sexes. In males, the percentage and absolute number of lymphocytes decreased somewhat. In females, the percentage of lymphocytes decreased slightly while the absolute number increased at LD and MD and was unchanged at HD.

Blood glucose increased in both sexes in a dose-related manner. Creatinine and urea were unchanged in males and slightly increased in females. Both sexes showed a decrease in albumin and consequently shifts in the ratio of albumin to globulins. Increases in the globulin levels were consistent with some kind of immune stimulation as suggested by the neutrophil shifts.
Triglycerides and cholesterol were increased in both sexes.
ALT and AST were increased in both sexes.

There was a non-significant increase in potassium in the HD females that was not seen in the males.

Summary of clinical chemistry findings: males

	Dose mg/kg			
	0	3	6	12
Glucose mM	3.40±0.40	3.73±0.20	4.40±0.43	4.81±0.21*
Albumin g/l	32.7±0.67	30.7±0.33	31.0±0.58	29.3±0.33*
Globulins g/l	23.7±0.88	25.3±0.67	29.3±0.88*	32.0±2.08**
Triglycerides mM	0.87±0.151	0.84±0.123	1.29±0.038*	0.93±0.038
Total choles mM	2.25±0.09	2.59±0.06*	2.99±0.13**	3.24±0.45
ALT IU/l	31±2.5	31±1.7	59±16.4	54±15.7
AST IU/l	72±4.7	77±3.5	114±11.0	100±11.2
K+ mM	5.33±0.27	4.73±0.29	5.23±0.27	4.57±0.09

Summary of Clinical Chemistry Findings: females

Glucose mM	4.87±0.44	5.47±0.51	5.64±0.15	5.89±0.05
Urea mM	4.50±0.47	6.60±1.89	5.15±0.72	5.15±0.57
Creatinine mM	31.0±1.00	32.2±1.45	34.3±2.03	33.7±0.88
Albumin g/l	36.3±0.33	31.7±0.88***	31.3±0.33***	31.3±0.33***
Globulins g/l	25.7±1.20	31.3±2.91	33.7±0.33*	32.0±0.58
Triglycerides mM	0.63±0.15	0.70±0.24	0.87±0.06	0.79±0.16
Total choles mM	1.89±0.26	2.42±0.34	2.46±0.14	2.32±0.03
ALT IU/l	19±1.0	26±2.8	52±21.7	57±18.5
AST IU/l	70±7.2	77±5.6	224±135	209±91.3
K+ mM	4.40±0.17	4.40±0.15	4.47±0.03	5.13±0.12

Organ Weights
Absolute and relative spleen weight was increased in males (dose-related)
Absolute kidney weight showed a dose-related decrease in females ($p<0.05$)
Absolute and relative adrenal weight was increased in the MD and HD ($p<0.01$) females

Relative weight of the adrenals was increased ($p<0.01$) in the LD males.
There was no consistent pattern in the reproductive organ weights of either sex.

Organ Weight Summary

Males				
	Dose mg/kg			
	0	3	6	12
Spleen(absol) g	0.56±0.01	0.63±0.06	0.68±0.04	0.79±0.09
Spleen (g/kg)	3.08±0.04	3.57±0.29	3.69±0.08**	4.26±0.46
Adrenals (mg/kg)	278±2.6	325±2.8***	240±25.5	284±51.2
Pituitary (mg/kg)	38.1±2.9	49.0±6.1	41.4	39.9±4.9
Females				
Kidney (absol) g	1.53±0.013	1.49±0.122	1.43±0.04	1.40±0.02**
Adrenals (absol) mg	58±2.4	47±3.1	66±1.5	83±3.9***
Adrenals (mg/kg)	360±26	292±30	415±10	561±32
Spleen (g/kg)	3.14±0.11	3.74±0.14	3.56±0.32	3.84±0.35
Pituitary (mg/kg)	57.4±1.8	49.1±6.6	58.6±7.3	60.5±3.4

***<0.1%

Histopathology was not done.

Study title: Preliminary toxicity study by intravenous infusion to CD rats for seven days

Key study findings: Beyond the effects of the vehicle, PCV and Hb were decreased in 20 mg/kg (HD m and HDf) and (10 mg/kg) MDf. It is not clear if changes in the hematology are entirely due to the irritation and damage to the injection sites and surrounding tissues. A slight decrease was reported in serum albumin in HD f as well as increases in AST and ALT in the same group. Consistent with the previous study there was poor local tolerability.

Study no.: DDO548

Conducting laboratory and location: Pharmaco LSR, Suffolk, England
Date of study initiation: September 15, 1994
GLP compliance: statement included
QA report: yes ( ) no (x)
Drug, lot #, and % purity: SR33589B, batch 92.01, purity >99%
Vehicle: mannitol, monosidic anhydrous phosphate, water for injection

Methods Sprague-Dawley rats (CD), 3/sex/group were given intravenous doses of 0(saline controls), 0(vehicle controls), 5, 10 or 20 mg/kg/day each day for 7 days. Drug was given via the femoral vein using a surgically installed catheter. After a "post-operative recovery period of a minimum of seven days" dosing began.
Observations:
Clinical signs: twice daily
Bodyweight (pre-study, day of surgery, start of treatment, day 3 after surgery, necropsy)

Food consumption: per week
Hematology, clinical chemistry: day 7
Euthanasia: gross observations, organ weights, tissues collected for histopathology

Statistical analysis: p values included in the tables are associated with the following statistical significance:

*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Results
There was no unscheduled mortality. One HD female was euthanized day 2 of treatment for loss of catheter patency and was replaced.
Clinical signs (the sponsor felt that these were associated with the infusion process but not the drug):

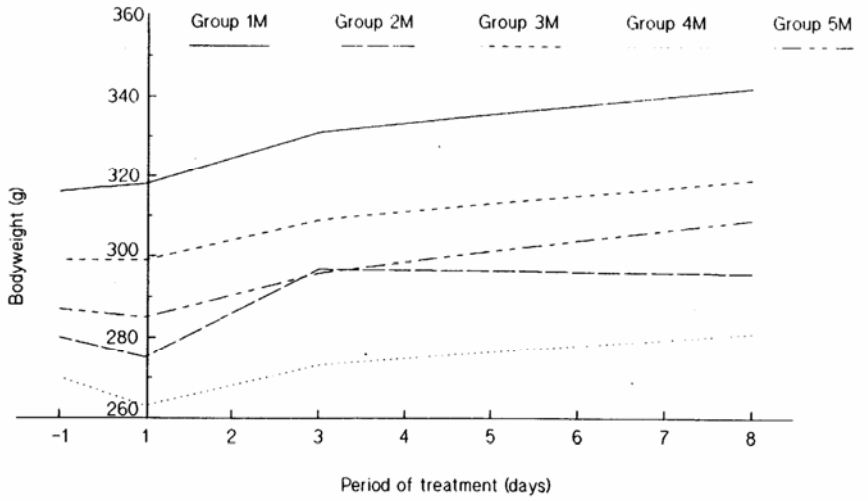
Arched back; several treated females, 1 sal control f, 2 HDm
Hunched, low activity; 20 mg/kg animals
Tremors: MD and HD females
Piloerection: HDf
Ptosis: MD and HD animals

Necropsy findings around the catheter tip showed visible damage to the veins and tissues.

Body weight and food consumption
The vehicle control in both sexes showed decreased body weight gain as did the HD females.

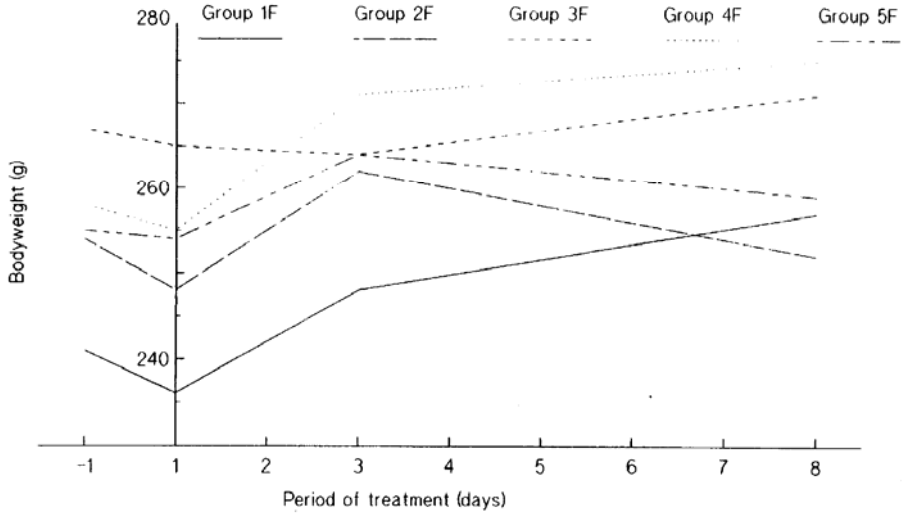
Group mean bodyweight versus period of treatment - males

Group	:	1	2	3	4	5
Compound	:	Saline Control	Placebo Control	----SR 33589B-----		
Dosage (mg/kg/day)	:	0	0	5	10	20



Group mean bodyweight versus period of treatment - females

Group	:	1	2	3	4	5
Compound	:	Saline Control	Placebo Control	----SR 33589B-----		
Dosage (mg/kg/day)	:	0	0	5	10	20



There were no consistent effects on food consumption.

Hematology and Clinical Chemistry

Hematology showed a decrease in PCV, Hb and RBC in the HDm and in a dose-related pattern in females. These effects were mild in the vehicle control groups.

Summary of hematology effects (mean ±SD)

Males					
	Dose mg/kg				
	saline	vehicle	5	10	20
PCV %	45±2	43±2	40±3	42±3	38±1
Hb g%	15.3±1.1	14.5±0.8	13.3±1.2	13.9±0.7	12.7±0.4
RBC mil/cm	7.95±0.49	7.35±0.25	6.80±0.91	7.26±0.58	6.54±0.31
Platelets 1000/cm	896±65	921±314	1110±102	928±245	1255±246
PT secs	18.3±4.6	14.1±0.2	14.2±0.4	13.7±1.0	14.1±1.0
Females					
PCV %	43±2	41±4	40±3	38±2	34±4
Hb g%	14.7±0.6	14.1±1.4	13.5±0.5	12.9±0.9	11.7±1.4
RBC mil/cm	7.64±0.74	7.64±0.52	6.68±0.25	6.56±0.47	6.38±0.81
Platelets 1000/cm	920±40	1172±147	1092±200	1174±155	1330±103
PT secs	13.4±0.1	13.4±0.4	14.0±0.6	14.4±0.5	13.5±0.6

There was a dose-related decrease in albumin and the A/G ratio in both sexes. There were mild increases in ALT and AST in females.

Summary of Clinical Chemistry effects (mean ±SD)

Males					
	Dose mg/kg				
	saline	vehicle	5	10	20
Albumin g%	2.8±0.2	2.2±0.6	2.0±0.9	1.9±0.3	1.9±0.2
P mmol/l	2.6±0.1	2.3±0.1	2.5±0.0	2.7±0.1	2.4±0.1
Females					
ALT IU/l	34±9	38±11	37±8	38±2	47±7
AST IU/l	107±20	118±21	122±30	101±16	142±40
Total chol mg%	79±11	64±10	49±11	53±31	49±11
Albumin g%	3.5±0.2	2.9±0.4	2.0±0.1	2.2±0.6	2.0±0.2
K+ mmol/l	2.9±0.3	2.9±0.2	2.9±0.1	2.8±0.3	3.2±0.4

Organ Weights

No trends were apparent in the absolute organ weights.
There were inconsistent increases in normalized lung and kidney weight in treated females.
Normalized spleen weight was also increased in both sexes although a steady dose-related response was not apparent.

Summary of organ weight effects (mean ±SD)

	Dose mg/kg				
	saline	Vehicle	5	10	20
Kidney (rel to body weight)	0.787±0.081	0.800±0.02	0.912±0.06	0.916±0.09	0.864±0.05
Lungs (rel to body weight)	0.498±0.06	0.609±0.05	0.541±0.03	0.692±0.12	0.615±0.04

Histopathology results were not provided.

Study title: Toxicity study by intravenous infusion to CD rats for four weeks

Key study findings: The text of this report notes poor weight gain and marked weight loss in several control animals. The jackets used to protect the catheters were irritating to the animals and the desire to escape from the jackets was so intense that 3 deaths were directly attributed to this. It was also reported that there were corneal opacities and keratopathies due to the period of anesthesia during the catheter implantation. Overall, the reviewer is left with the impression that the animals were stressed, in poor condition, and that the overall value of the study must be questioned.
Intravenous infusion of all doses was associated with a higher incidence of abscesses and/or hematomas than caused by vehicle alone: vehicle 6/20 (no deaths); LD 12/20 (2 deaths), MD 17/20 (3 deaths), HD 18/20 (4 deaths). Hematology also showed dose-related anemia in the males but only in HDf. Increased WBC counts (primarily neutrophilia and monocytosis) as well as decreased total cholesterol and albumin were seen in both sexes. AST and ALT showed dose-related increases in males.

Study no.: TSA0963

Conducting laboratory and location: Pharmaco L.S.R., Suffolk, England
Date of study initiation: November 14, 1994
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589B (batch 92.01), purity
Vehicle: mannitol, monosidic anhydrous phosphate, water for injections
Methods: Sprague-Dawley rats (10/sex/group) were given either saline (control), vehicle (vehicle control) or intravenous doses of 2,4 or 8 mg/kg/day for 28 -29 days. Injections were made into an indwelling catheter installed in the femoral vein.
Observations:
Body weight: Pretreatment, day 3 after surgery, start of treatment, twice weekly during treatment, at necropsy
Food consumption: Mean consumption per rat calculated for each group per week
Ophthalmoscopy: pretreatment, and after 21 days of treatment the saline, vehicle controls and HD groups were examined
Hematology and clinical chemistry: blood collected day 23
Urinalysis: between days 25-28, urine collected from all rats in individual metabolism cages

Toxicokinetics: day 29, samples collected from 2 rats/sex/group immediately before infusion, immediately after infusion, 2 hrs and 5 hrs after end of infusion

Euthanasia: gross observation, organ weights, tissues preserved for histopath
Electron microscopy: samples of liver and kidney taken from 3 males and 3 females per group

Statistical analysis: p values included in the tables are associated with the following statistical significance:

*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Results

Unscheduled mortality was seen in the drug-treated animals:

Saline control: 1 female, catheter problem
Vehicle control: 1 female, catheter problem; 1 male
LD: 2 males
MD: 2 males, 2 females Death of the 10 dosed animals was reported to be related to the
HD: 2 males, 2 females irritancy of the infusion.

Clinical signs: were reported for the premature decedents and included swollen abdomen, hunched posture, piloerection. Necropsy of the premature decedents showed:

> Necropsy revealed that all of the above animals had an adhesive mass at the infusion site, often involving the surrounding tissues and associated with inflammation and haemorrhage. The masses were diverse in appearance, and were identified at histopathology as either haematomas, consisting of a cystic space containing gelatinous material and clotted blood, or as abscesses which were paler in appearance and contained purulent material. Other common macroscopic findings included pallor of the internal organs, free and/or clotted blood in the abdomen, swollen spleen and enlarged lymph nodes. Histopathology also indicated changes in the liver, lungs, kidneys, spleen and lymph nodes of most of these animals.

The text states that few behavioral changes were associated with the other animals. These signs, reported almost exclusively for the HD group, included piloerection, decreased activity, hunched, ptosis, ataxia, pallor and slow respiration following dosing during the third week.

Body Weight and Food Consumption

There were no apparent effects on body weight gain and food consumption. However, the text of the report notes that mean weight gain in the HD females was relatively high compared to the saline and vehicle controls. The sponsor attributes this to the poor weight gain of the female control animals and marked weight loss in 2 female saline control animals. It was also noted that the jackets worn to protect the catheters were very irritating to the rats and led to the death of 3 animals. While the HD groups gained more than the control groups, food consumption was decreased in the HD animals.

Changes are summarized below.

Summary of body weight changes (Mean±sd)

Males					
	Dose mg/kg/day				
	saline	Vehicle	2	4	8
Day 0	289±44	318±18	305±27	326±15	310±21
Day 28	370±64	391±29	388±24	399±24	399±36
Change day 0-28	81±39	74±18	75±12	71±33	90±26
Food consumption: week 4	170±39	187±22			159±27
Females					
Day 0	247±23	247±17	253±8	249±10	247±14
Day 28	282±27	279±31	294±17	287±16	297±20
Change day 0-28	34±10	31±19	41±18	40±16	51±25*
Food consumption: week 4	148±32	146±24			139±19

*p<0.05

Ophthalmoscopy: The sponsor states that there were no treatment-related findings. But it was also noted that there were “superficial corneal opacities” and “bands of keratopathy” considered to be associated with the period of anesthesia during the surgery.

Summary of ophthalmic findings post-surgery and pre-treatment

	males			females		
	saline	Vehicle	HD	saline	Vehicle	HD
Corneal opacities	5	5	7	10	7	10
keratopathy±ulcer	4	5	7	2	2	3
Ulcer	0	1	0	1	0	0

Hematology and Clinical Chemistry

A dose-related increase in neutrophils was seen in both sexes. This is consistent with the other intravenous dosing studies and is most likely related to the tissue damage done by the irritancy of the injected material, stress and subsequent health complications.

A decrease in PCV, Hb, RBC was seen in both sexes. In the females, part of the decrease was due to the vehicle. This vehicle effect was not seen in the males.

Summary of hematology mean±SD

Males					
	Dose mg/kg				
	saline	vehicle	2	4	8
PCV%	44±15	44±3	37±10^{by}	35±3^{cy}	35±3^{by}
Hb g%	15.4±0.8	15.3±1.1	12.7±3.8^{by}	12.2±1.1^{cy}	12.1±1.1^{cy}
RBC mil/cm	8.27±0.36	8.24±0.49	6.99±1.96^{by}	6.99±0.53^{by}	7.06±0.30^{ax}
Females					
PCV%	42±3	38±6^{a}	38±3^{a}	38±3^{a}	31±4^{cy}
Hb g%	14.8±0.9	13.2±2.2^{a}	13.3±1.1^{a}	13.0±1.4	10.3±1.3^{cz}
RBC mil/cm	7.74±0.48	7.00±1.17^{a}	7.01±0.48^{a}	6.99±0.64^{a}	5.92±0.80^{cy}

Significant when compared with saline control: a=p<0.05, b=p<0.01, c= p<0.001
Significant when compared with vehicle control: x=p<0.05, y=p<0.01, z=p<0.001

In males, dose-related increases were seen in AP, ALT and AST. Significant increases were also seen in GGT. A dose-related decrease in total cholesterol was also apparent. Total protein was also significantly decreased as was albumin and the A/G ratio. Potassium was significantly increased at the HD: saline 3.6±0.2 mmol/l; vehicle 3.9±0.4; LD 3.7±0.2; MD 3.9±0.7; HD 4.2±1.0 (p<0.05).

The results in females were very similar to the males. Only the tables of male results will be shown.

Blood chemistry - group mean values after 3 weeks of treatment

Group	:	1	2	3	4	5
Compound	:	Saline Control	Placebo Control	---	SR 33589B	---
Dosage (mg/kg/day)	:	0	0	2	4	8

Group / sex	AP iu/l	ALT iu/l	AST iu/l	GGT iu/l	Urea mg%	Creatinine mg%	Glucose mg%	Total Bilirubin mg%	Triglycerides mg%	Total Cholesterol mg%
1M	145	44	105	1	31	0.6	87	0.1	55	65
SD	22	10	16	1	4	0.0	8	0.0	12	12
2M	167	42	106	1	30	0.6	88	0.1	53	69
SD	115	7	18	1	7	0.1	13	0.1	20	14
3M	148	89	267	2^{x}	39	0.6	93	0.2^{b}	46	54^{x}
SD	55	146	407	2	20	0.1	12	0.1	16	18
4M	166	139	310	2^{x}	33	0.6^{x}	92	0.2^{b}	49	47^{ay}
SD	31	171	348	1	8	0.1	12	0.0	13	17
5M	176	197^{ax}	480^{ax}	2^{x}	37	0.6	77^{x}	0.2^{cx}	51	46^{by}
SD	37	201	427	1	6	0.1	14	0.1	9	14

SD Standard deviation.
Significant when compared with Group 1: a - p<0.05; b - p<0.01; c - p<0.001.
Significant when compared with Group 2: x - p<0.05; y - p<0.01.

Urinalysis: No trends were obvious in the data presented.

Organ Weight

The same organs affected after oral administration were also affected after intravenous administration.

Summary of organ weight changes

		males			females		
		2mg/kg	4mg/kg	8mg/kg	2mg/kg	4mg/kg	8mg/kg
Brain	Absolute	↑	↑	↑			
Adrenals	Absolute	↑	↑	↑			↑
	normalized	↑	↑	↑			↑
Heart	Absolute		↑	↑			
	normalized				↓	↓	↓
Kidneys	Absolute	↑	↑	↑	↑	↑	↑
	normalized	↑	↑	↑	↑	↑	↑
Liver	Absolute			↑			↑
	normalized			↑			↑
Lungs	Absolute						
	normalized			↑	↓	↓	
Ovaries:	absolute						↑
Uterus+cervix:	absolute						↑
	Normalized						↓
Prostate	absolute	↑	↑	↑			
	normalized	↑	↑	↑			
Seminal	absolute		↓	↓			

Vesicles	normalized		↓	↓			
Spleen	absolute	↑	↑	↑		↑	↑
	normalized	↑	↑	↑		↑	↑

Gross and Histologic Findings

Under macroscopic observations the following items were found for males:

Summary of macroscopic findings

	males				
	sal	veh	LD	MD	HD
Testes appear small	0	0	0	0	1/8
Liver masses	0	0	0	4/8	4/8
Infusion site masses	0/10	2/10	4/10	9/10	9/10
Spleen swollen	1	0	0	4/10	5/10

Under macroscopic observations the following items were found for females:

	females				
	sal	veh	LD	MD	HD
Ovaries markedly enlarged	0	0	0	0	1/8
Liver masses	0	0	0	1/8	4/8
Infusion site masses	1/10	6/10	8/10	8/10	9/10
Spleen swollen	1	1	0	4/10	4/10

Alveolar macrophage accumulations were reported in the lungs of 2 HD males and 1 LD female.
Testicular degeneration (germinal epithelium): 1LD

Histologic findings in the liver

	males					females				
	Sal	veh	LD	MD	HD	Sal	veh	LD	MD	HD
Periacinar necrosis+fibrosis	0	0	0	0	4^{ax}	0	0	0	1	5^{by}
Focal necrosis	0	0	0	0	3	1	1	1	0	0
Necrosis of individual hepatocytes	0	0	0	0	2	0	0	0	0	2

Significant when compared with saline control: a= $p<0.05$, b=$p<0.01$
Significant when compared with vehicle control: x=$p<0.05$, y=$p<0.01$

Discrepancies between text incidences and table incidences were evident.

4-Week intravenous infusion toxicity study in the rat: toxicokinetic data. Complement to the report RS0006950316/01. TSA0963

This report contained the TK data mentioned in the previous study report. Blood samplings were collected day29 immediately before infusion, immediately after infusion, 2 and 5 hours after infusion from 2 rats/sex/sampling time in the treated groups. SR33589 and SR35021 in the plasma were analyzed by HPLC with UV detection.

SR35021 was not detected in either sex. The levels of SR33589 found in the LD group of both sexes was at or below the limit of quantitation and at the final timepoint below the limit of detection. High background levels were reported for the MD and HD groups of both sexes. This is summarized in the reviewer's table below.

Limit of quantitation: 0.020 mg/l
Limit of detection: 0.010 mg/l

Summary of mg/ml SR33589

Relative sampling time (hrs)	males		Females	
	MD	HD	MD	HD
0	0.0455	0.1460	0.0240	0.1275
1.34-1.43	0.0385	0.1690	0.0765	0.0915
3.06-3.13	0.0265	0.1925	0.0325	0.0770

The plasma profiles did not allow assessment of pharmacokinetic parameters.

Study title: Two-week oral toxicity study in the dog

Key study findings: There were indications of alterations in renal (increases in serum creatinine, changes in sodium and potassium excretion, changes in urinary pH) and hepatic function (slight increase in AST, ALT ≥60 mg/kg and histologic findings of inflammatory cell infiltrates in bile ducts). A NOAEL for the renal changes was not identified with serum creatinine increased in both sexes ≥25 mg/kg. Both sexes also showed dose-related decreases in sodium excretion. First degree heart block was seen (≥25 mg/kg) and attributed to the drug as was an increase in QT and QTc (≥60 mg/kg). Prolongation of the PR interval was seen in both sexes (≥25 mg/kg) but was more consistent in the males. Both absolute and normalized prostate weight of all drug-treated males was increased over the control values. Interestingly, the same was true of the uterine weights of the female drug-treated dogs. Ovarian weight was increased in the MD

and HD groups. Due to the seasonally monoestrus cycles of female dogs one would not expect to see any particular pattern in the reproductive tract. Signs of vomiting, diarrhea and ptyalism were seen ≥60 mg/kg. Findings of darkened liver, inflammation of the biliary tract and greenish colic or rectal contents suggest that the GI tract as well as the heart, reproductive tract, liver and kidneys is also a target for drug-associated toxicity.

Study no.: TSA0883

Conducting laboratory and location: Sanofi Recherche, Montpellier Cedex, France
Date of study initiation: March 31, 1993
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589B, batch 92.01
Given orally in gelatin capsules. Empty capsule used as control.

Methods Beagles, 3/sex/dose approximately 1 yr old, were given daily oral doses of 0, 25, 60 or 140 mg/kg for 15-16 days.
Clinical signs: 2x/day
ECG: days -7 and 10, 2-4 hours after dosing. Leads I, II and III, aVR, aVL, aVF (Bazett's)
Ophthalmoscopy: days -2 and 8
Body weight: pre tx and then weekly
Food intake: daily
Hematology, clinical chemistry: days -9 and 14 from fasting animals
Urinalysis: on overnight samples days -5 and 10, collection method not disclosed
TK: blood collected from all animals day 15 at 1,2,4,8 and 24 hours after dosing
Necropsy: gross observations, organ weights, tissues collected for light microscopy

Statistical analysis: p values included in the tables are associated with the following statistical significance:

*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Results

No unscheduled mortality was reported.

Clinical signs: reported for ≥60mg/kg/day were vomiting, ptyalism, diarrhea. All described as dose related and related to drug treatment. Vomiting was seen primarily in the first 3 days. Ptyalism was noted from day 4 onwards

ECG The sponsor came to the conclusion that increased QT and AV block were drug-related. This finding may be expected from the pharmacology.
1st degree AV block:

LD: 1m, 1 f
MD: 1m, 1f
HD: 2m, 2f

Increased QT
LD: 1m, 1f
MD: 1m, 2f
HD: 2m, 3f

Heart rate decreased in males but not in a totally consistent or dose-related pattern. PR interval was increased, QTc was increased and T amplitude was increased.

Summary of ECG effects: Males

	Dose mg/kg			
	0	25	60	140
P (0.01s)	4.5±0.20	4.3±0.87	5.0±0.53	5.3±0.24
PR (0.01s)	9.3±1.17	12.5±2.02	12.0±1.16	14.7±1.50
QT(0.01s)	18.8±0.09	23.3±1.15	22.2±0.63*	23.8±1.08*
T amplitude mv	0.166±0.1449	0.135±0.0675	0.466±0.0983	0.241±0.0015
QTc	8.44±0.31	8.11±0.62	9.22±0.27	9.52±0.42

PR interval was increased in a non-dose-related pattern as was T amplitude. QT and QTc were increased also.

Summary of ECG effects: Females

P (0.01s)	4.9±0.39	5.4±0.07	5.0±0.36	5.8±0.12
PR (0.01s)	11.8±0.47	13.5±1.85	12.1±0.50	15.0±0.65
QRS (0.01s)	4.8±0.73	6.2±0.54	5.2±0.41	7.0±0.52
QT(0.01s)	20.4±0.30	21.2±1.10	21.6±1.00	26.4±2.16
T amplitude mv	0.192±0.1058	0.211±0.0491	0.200±0.0333	0.208±0.350
QTc	8.34±0.805	8.77±0.593	9.32±0.241	9.54±0.335

Ophthalmoscopy: 1 HD male showed visible anterior suture lines on the lens and a "slight micropapilla in the tapetal fundus" of both eyes.

Body Weight and Food Intake
All animals seemed to lose some weight during the study with a dose related trend apparent.

Control males lost 4% of baseline while MD males lost 9% and HD males lost 15%.
Control females lost 3% of baseline while LD lost 6%, MD lost 11%, and HD lost 16%.

Food intake also decreased in a dose-related manner.

Hematology and Clinical Chemistry

No consistent trends were apparent in the hematology.

Increased ALT, AST was seen in treated males as well as a decrease in K (HD only). Serum K was decreased in HD females also. Creatinine was increased in both sexes.

Summary of clinical chemistry effects: Males

	Dose mg/kg			
	0	25	60	140
Creatinine mcM	69.7±2.91	72.0±3.51	83.0±3.21	77.0±4.04
Tbili mcM	1.9±0.17	1.7±0.06	1.5±0.43	3.7±1.15
ALT IU/l	20±0.6	42±9.4	68±17.8	56±12.9
AST IU/l	19±1.9	18±2.2	35±15.3	43±22.2
Potassium mM	4.33±0.186			3.87±0.260
P mM	1.37±0.06	1.29±0.094	1.19±0.107	1.13±0.179

Summary of clinical chemistry effects: Females

Dose mg/kg	0	25	60	140
Creatinine mcM	67.7±1.33	68.3±3.71	80.0±5.69	74.00±3.00
Tbili mcM	2.4±0.049	2.0±0.17	2.4±0.09	3.0±0.41
ALT IU/l	31±2.0	25±2.6	62±14.0	36±3.5
AST IU/l	23±6.9	19±5.3	27±5.2	26±6.7
ALP IU/l	167±23.3	126±25.8	68±13.0*	58±0.6**
P mM	1.17±0.026	1.27±0.091	1.21±0.064	1.04±0.027

Urinalysis

Decreased urine pH was seen in both sexes. An apparently dose-related decrease in sodium excretion was seen in both sexes with changes in the ratio of excreted sodium to potassium. The changes persisted when corrected for total volume excretion. In both sexes there were dose-related decreases in creatinine and potassium excretion.

Summary of urinalysis results

Males				
	Dose mg/kg			
	0	25	60	140
pH	6.7±0.17	6.2±0.17	6.0±0.00*	5.3±0.33*
Na mM	136±29.8	108±45.0	40±16.5	32±12.8
Females				
pH	7.2±0.17	6.0±0.29*	5.7±0.17*	5.7±0.44*
Na mM	173±53.1	167±17.3	58±19.2	21±9.7*

*p<2.4%

Organ weight effects

Male organ weight summary

	0	25mg/kg	60mg/kg	140 mg/kg
Liver (absolute + normalized)		↓	↓	↓
Spleen (absolute + normalized)		↓	↑	↑
Heart (absolute and normalized)		↑	↑	↑
Lungs		↓	↓	↓
Prostate absolute	5.34±1.103	8.79±0.98	8.33±0.52	8.26±1.38
Prostate normalized	0.45±0.056	0.72±0.071	0.79±0.062	0.71±0.124

Female organ weight summary

	25mg/kg	60mg/kg	140 mg/kg
spleen (absolute + normalized)	↓	↓	↑
Adrenals (absolute + normalized)	↑	↑	↑
Thymus (absolute + normalized)	↓	↓	↓
Lungs (absolute + normalized)	↓	↓	↓
Pituitary (absolute + normalized)	↑	↓	↓

Data from the female reproductive tract were striking and are shown below. There was no explanation apparent for the increased uterine and ovarian weights.

Summary of female reproductive organ data

Dose group (mg/kg)	Uterus		Ovaries	
	Absolute wght (g)	Normalized wght (g/kg)	Absolute weight (mg)	Normalized wght (mg/kg)
0	4.653±1.7342	0.437±0.1285	1046±144.3	102±8.4
25	5.433±0.9309	0.572±0.0994	1023±154.9	108±16.1
60	15.011±2.592	1.375±0.1402	1746±221.6*	161±9.6*
140	17.019±4.5153*	1.876±0.5504	1486±85.4	161±7.9*

*p<1.6%

Histopathology Findings

Summary of female findings

	0	25mg/kg	60mg/kg	140mg/kg
Mammary hyperplasia	2	2	3	3
Mammary interstitial edema	0	0	3	1
Cystic corpus luteum	0	0	1	1

Summary of findings from both sexes

	0		LD		MD		HD	
	m	f	m	f	m	f	m	f
Chronic inflammatory cell infiltrate of several biliary ducts	0	0	0	0	1	2	1	1
Single necrotic focus (liver)	0	0	0	0	0	1	0	0
Mesenteric lymph nodes: foamy	0	0	0	0	0	0	3	1
Mesenteric lymph nodes+Sudan black inclusions	3	0	2	2	2	2	3	3
EM: lamellar inclusions in hepatocytes	0/6		0/6		3/6		0/6	
EM: myelin-like inclusions in hepatocytes	0		0		0		4/6	

Study title: 3-month oral toxicity study in the dog

Key study findings: Target organs of toxicity were demonstrated to be the digestive tract, heart and thyroid. Heart rate was decreased only in HD males. PR interval showed a dose-related increase in males but was increased only in HDf. T amplitude showed an increase in all drug-treated groups. First degree AV block was seen in 2 males and several times in 1 female. QTc was increased day 93 (≥17.5 mg/kg) in both sexes. There was also a slight prolongation of thrombin time in both sexes (≥5 mg/kg) and liver transaminases (females, 60 mg/kg). Mild decreases in cholesterol levels (HDm only; females: dose-related) possibly indicate some kind of liver involvement. T3 values showed dose-related decreases in both sexes. T4 showed non-dose-related decreases in both sexes.
Urinalysis showed decreased creatinine excretion(males ≥17.5 mg/kg; females: ≥5 mg/kg). Sodium and potassium excretion were decreased in males (≥5 mg/kg). Potassium excretion was decreased in females ≥17.5 mg/kg.

Lung weight showed an increase (50%) in females only. Uterine weight was inconsistently increased in all drug-treated groups. Testes weight was increased in all drug-treated groups.

Study no.: TXC0886

Conducting laboratory and location: Sanofi Recherche, Montpellier Cedex, France
Date of study initiation: June 24, 1993
GLP compliance: statement included
QA report: yes ( ) no ( )
Drug, lot #, and % purity: SR33589B batch 92.01
Administered in gelatin capsule. Empty capsule used as vehicle

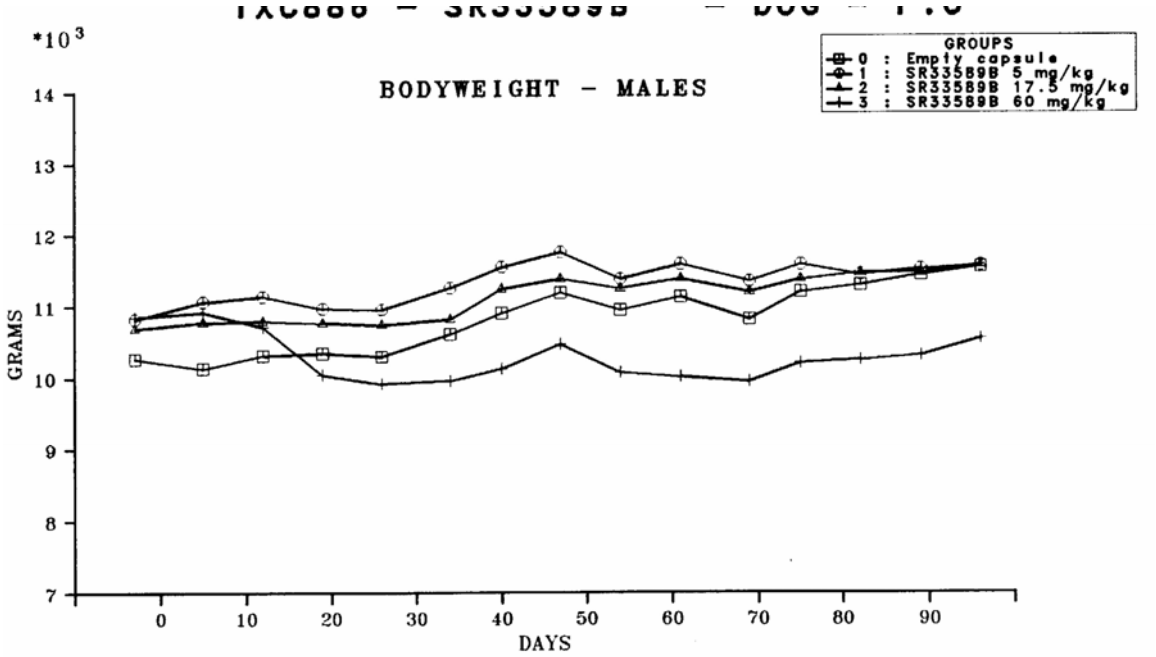
Methods Beagles approximately 1 year old (4/sex/dose) were given doses of 0, 5, 17.5 and 60 mg/kg/day for 97-99 days.
Clinical signs: 2x/day
ECG: days -2, 29, 93 from 2-4 hours after dosing. Leads I, II, III and aVR, aVL, aVF
Ophthalmoscopy: days -2, 85
Body weight: weekly
Food consumption: daily measurement.
Hematology and clinical chemistry: days -7, 30 and 85; day 89 for coagulation
Lymphocyte subpopulations: day 85
Hormone: T4, T3 day 89
Urinalysis: days -2 and 84, overnight samples collected by unknown method
TK: taken days 35 and 97 pre-dose and 1,2,4 and 6 hours after dosing.

Statistical analysis: p values included in the tables are associated with the following statistical significance:

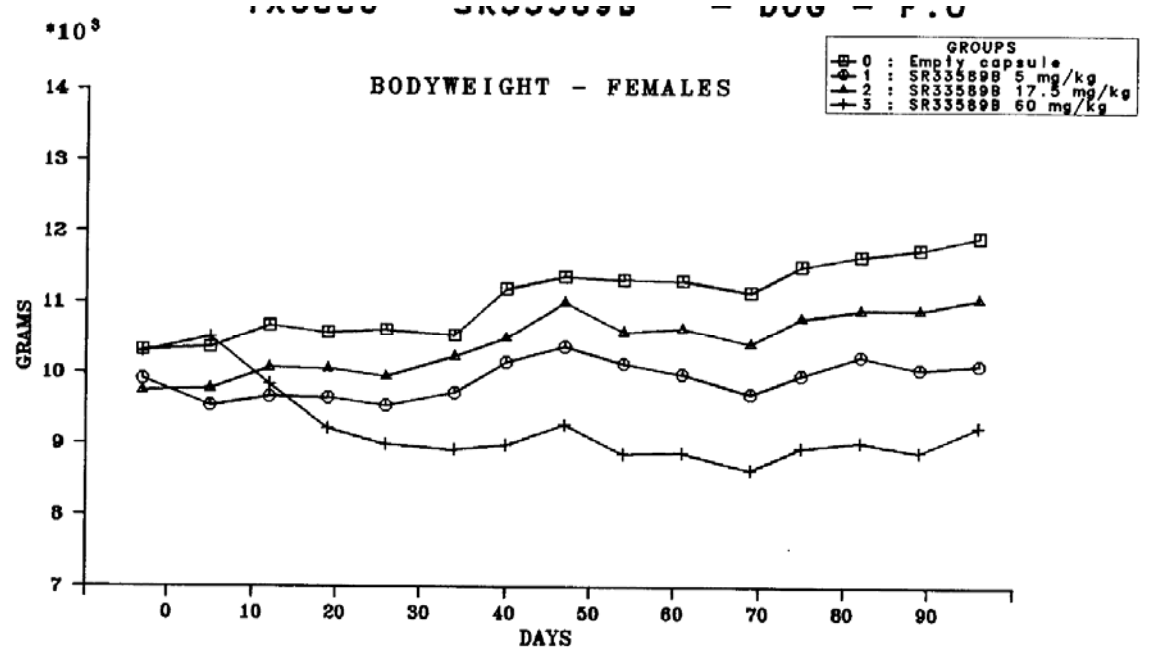
*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Results
No unscheduled mortality was reported.
Consistent with the 2 week study, reported signs were ptyalism, diarrhea and vomiting. No histologic correlates were reported. Frequency of observations increased with increasing dose.

The HD males showed a decrease in body weight. Food intake was also decreased in this group.



HD females lost weight over the study. The other treated females showed decreased gain. Food consumption was consistently affected only in the HD group.



ECG:
There were inconsistent heart rate effects between males and females.

P duration was increased in females. PR interval was increased in both sexes and in some cases was reported to lead to first degree AV block (2 males on day 29 and 1 female on 2 separate days). QT interval was significantly increased in both sexes. QTc, calculated by Bazett's formula did not show statistically significant differences. RR interval was increased with treatment in both sexes also. T wave amplitude was increased in both sexes.

Summary of ECG effects

Parameter	Dose mg/kg			
	0	5	17.5	60
Males				
HR bpm day 29	111.6±8.9	114.5±11.8	100.0±9.3	95.3±6.8
Day 93	104.9±9.6	102.0±9.3	101.3±8.0	86.4±18.8
PR(0.01s) day 29	9.1±0.9	9.5±0.3	9.3±0.3	13.8±0.4**
Day 93	8.9±0.8	9.5±0.4	9.9±0.7	11.9±0.8
T amplitude mV day 29	0.066±0.05	0.093±0.09	0.228±0.03	0.271±0.08
Day 93	-0.013±0.09	0.141±0.16	0.173±0.04	0.302±0.08
Females				
P (0.01s) Day 93	4.7±0.37	4.8±0.52	4.0±0.13	6.2±0.4*
PR (0.01s) day 29	10.5±0.55	10.4±0.74	9.1±0.69	11.8±0.79
Day 93	10.8±0.66	10.2±0.92	9.5±0.51	12.7±1.22
T amplitude mV day 29	0.090±0.09	0.334±0.09	0.179±0.05	0.183±0.13
Day 93	0.040±0.15	0.140±0.11	0.203±0.07	0.430±0.15

*<4.1%

Summary of QTc effects

Parameter	Dose mg/kg			
	0	5	17.5	60
Males				
QTc day 29	9.04±0.34	8.93±0.27	8.45±0.34	8.71±0.41
Day 93	7.18±0.29	7.55±0.27	8.12±0.31	8.22±0.90
Females				
QTc day 29	7.62±0.39	8.26±0.34	8.76±0.05	8.60±0.37
Day93	7.22±0.23	8.05±0.28	8.39±0.38	8.60±0.38

Hematology and Clinical Chemistry

There were no findings of biological significance in the data as presented. A very unusual finding was the marked increase in basophils seen in the HD males on day 30(27% vs 0% in all other groups). This finding was not repeated on day 85.

There was a slight increase in thrombin time in drug-treated males and females on day 89.

Thrombin time Day 89

	Dose mg/kg			
	0	5	17.5	60
Males Thrombin time (s)	15.7±0.15	20.0±0.65**	20.3±0.08**	19.5±1.06**
Females Thrombin time (s)	16.1±0.68	18.7±0.73*	18.7±0.53*	19.0±0.53*

*p<0.3.4%, **p<0.8%

In both sexes there was a slight shift to greater numbers (%) of T cells and decreased B cell counts. The absolute counts did not change.

There was a slight decrease in total cholesterol in the HD males at 30 and 85 days. The females showed a dose-related decrease in total cholesterol even before dosing started. Likewise phospholipids also showed a decrease in females. HD females showed an increase in ALT at 30 days and 85 . Both sexes showed a decrease in T3 at the HD. T4 was also decreased in HD females (T3/T4 ratio also decreased in this group).

Summary of Clinical Chemistry Findings: Males

	Dose mg/kg			
	0	5	17.5	60
Cholesterol (mg/dl) day 30 day 85	 3.05±0.278 3.10±0.300			 2.86±0.055 2.56±0.127
T3 nM day 89	1.48±0.151	1.21±0.011	1.36±0.084	0.93±0.077**
T4 nM day 89	36.84±3.569	26.64±1.303*	32.11±1.730	27.44±2.609

Summary of Clinical Chemistry Findings: Females

	Dose mg/kg			
	0	5	17.5	60
Cholesterol (mg/dl) Day -7 Day 30 Day 85	 4.45±0.318 4.55±0.327 5.03±0.679	 3.83±0.300 4.23±0.256 3.79±0.251	 3.66±0.400 4.15±0.871 3.60±0.385	 3.54±0.456 3.17±0.307 2.85±0.375
Phospholipids (mM)	5.59±0.42	4.54±0.226	4.33±0.383	3.46±0.465**
ALT (IU/l) day 30 Day 85	24±4.2 22±2.8			33±1.5* 80±51.0
AST (IU/l) day 85	18±3.4			41±19.2
T3 nM day 89	1.60±0.105	1.44±0.192	1.27±0.246	0.64±0.097**
T4 nM day 89	43.18±4.103	37.69±5.273	40.27±4.242	24.47±1.942*

The concentration of creatinine excreted decreased in both sexes. The ratio of sodium to potassium excretion increased in females. The volume of excreted urine showed a decrease in males and an increase in females.

Urinalysis results

Males				
	Dose mg/kg			
	0	5	17.5	60
Creatinine mM day 84	18.7±0.81	18.5±0.67	13.2±1.95*	14.7±1.60
Excreted urine volume ml	137.5±10.31	130.0±17.32	150.0±37.64	96.7±33.33
Sodium µM	31060±4771	29400±2403	25513±1573	23193±8786
Potassium µM	31959±2818	31584±3600	19965±2697	22880±8020
Females				
Creatinine mM day 84	15.7±1.82	11.9±0.46	10.0±1.53	14.8±1.87
Na/K urine concentration	0.95±0.063	1.02±0.171	1.18±0.141	1.32±0.138
Excreted urine volume ml	112.5±13.15	136.7±21.86	190.0±20.82*	140.0±5.77
Potassium µM	30429±2985	33626±6776	24729±6858	21771±6615

Organ weights

Normalized heart weight was slightly increased in the HD groups of both sexes. Normalized testes and prostate weight showed a dose-related increase. A U-shaped effect was seen in the normalized uterine weights.

Organ Weight Summary

Females				
	Dose mg/kg			
	0	5	17.5	60
Uterus g	4.172±0.7004	4.651±0.3927	9.621±4.0399	3.568±0.5642
Uterus g/kg	0.359±0.0413	0.493±0.0540	0.880±0.3553	0.407±0.0661
Lungs g/kg	6.72±0.398			9.06±1.032
Heart g/kg	7.57±0.452			10.76±0.388***
Males				
Testes g/kg	1.387±0.1337	1.466±0.1157	1.529±0.2611	1.535±0.1570

The sponsor provided the following summary of histopathology findings. The reviewer adds no others from the data provided.

Summary of histopathology findings

	0		5		17.5		60	
	m	f	m	f	m	f	m	f
Thymic involution	4	4	4	4	4	4	4	4
Pancreatic serous dedifferentiation							4	3
Decreased subcutaneous adipose tissue								2
Atrophic changes								1

The thymic involution was perceived as most likely physiologic with the exception of the HD group where there was an "increase in the intensity of this change…and its association with other modification might indicate a slight treatment-related stress."

Plasma levels of both compounds were below the limits of detection at the lowest dose. At the MD and HD, the plasma levels of parent compound exceeded those of SR35021.

AUC_{0-24} SR33589 (mg.hr/l)

Dose	Day 35			Day 97		
mg/kg/day	5	17.5	60	5	17.5	60
M	-	2.34	47.7	-	2.57	35.35
F	-	2.59	52.71	-	3.73	44.15
AUC_{0-24} SR35021 (mg.hr/l)						
M	-	0.191	2.991	-	0.408	2.461
F	-	0.347	4.694	-	0.376	3.240

Study title: One-year oral toxicity study in the dog

Key study findings: There were no findings in this study that raised new safety issues . In fact, some of the clinical chemistry changes seen in shorter duration studies were minimal to non-existent here. HR was slightly decreased in the HD groups of both sexes first noted at 93 days and persisting to the end of the study. QTc was increased in males ≥5 mg/kg and females ≥45 mg/kg. Non-significant increases in PR were seen in both sexes, less consistently in the females. Heart weight was increased in the HD animals of both sexes. The sponsor felt that the ECG changes might be related to the change in heart weight, but this is unclear to me as the ECG effects were similar to those of studies with no recorded change in heart weight. Serum and urinary electrolyte changes were still apparent. There were few findings of histological significance.
Changes in thyroid function were manifested by decreased T3 levels (45 mg/kg from 3 months of treatment; ≥15 mg/kg in males at 6 and 12 months; ≥5 mg/kg in females at 12 months). No trend was discernible in the lymphocyte subpopulations. In the lymphoblastic transformation studies, there were decreases in cpm primarily at MD and HD with corresponding decreases in standard error of the mean.

Con A
Females: Day 184: HD caused ~50% decrease in cpm with ~50% decrease in the SEM
Day 359: decrease at HD in 1 mitogen concentration
Males: day 184 no consistent effect
Day 359 decrease in cpm and SEM at MD and HD

PHA
Females: day 184 HD caused decrease in cpm and sem
Day 359 inconsistent decreases across the drug-treated groups

Males: day 184 decrease at MD and HD in cpm and sem
Day 359 decrease at MD and HD
PWM
Females: day 184 decreased at HD
Day 359 decrease at HD
Males :day 184 decreases at MD and HD but not dose-dependent. SEM decreased about 75% from the vehicle values.
Day 359 : decreases at MD and HD

Study no.: TXC0970

Conducting laboratory and location: Sanofi Recherche, Montpellier Cedex, France
Date of study initiation: October 19, 1995
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589B, batch 5SNP 505 Identity of molecule in capsule checked monthly during study.
Administered in gelatin capsules. Vehicle control was an empty capsule

Methods:
Beagles, approximately 1 year old at the start of the study, 5/sex/group, were given doses of 0, 5, 15, 45 mg/kg/day for 368 to 372 days.
Clinical signs: 2x/day
ECG days -3, 93, 187 and 364 before dosing . Leads I, II, III and aVR, aVL, aVF
Body weight: pre-tx then weekly
Hematology and clinical chemistry: samples collected days -7, 90, 181, 365
Lymphocyte subpopulations: blood collected days -7, 181, 365
IgG, IgM, hormones: performed on frozen samples collected on same days as for clinical chemistry and hematology
Lymphoblast transformation test: days -10, 184, 359 (concanavalin A (conA), phytohemagglutinin (PHA) and pokeweed mitogen (PWM). Cell proliferation measured after 4 days of incubation by measuring ^{3}H-thymidine incorporation.
Urinalysis: days -1, 86, 177, 363 on overnight urine samples, collection method unspecified
Toxicokinetics: samples collected days 30, 188, 366 pre-dose, 1,2,4 and 6 hours after treatment
Necropsy: euthanized days 369-373. gross observations made, organ weights, light microscopy
Statistical results: *1.0%<p≤5.0%; **0.1%<p≤1.0%; ***p≤0.1%

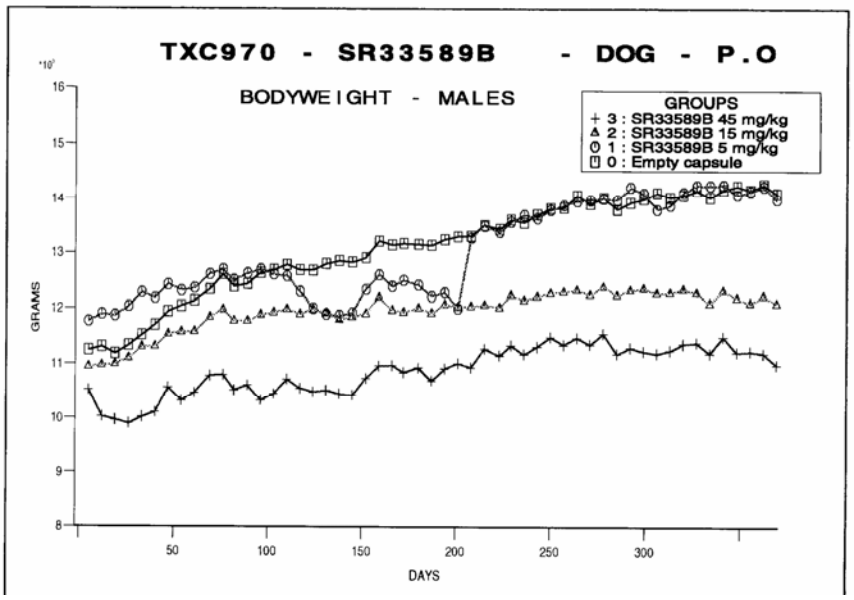
Results
Signs
Ptyalism 1/3 HD females
Vomiting: both sexes, ≥5 mg

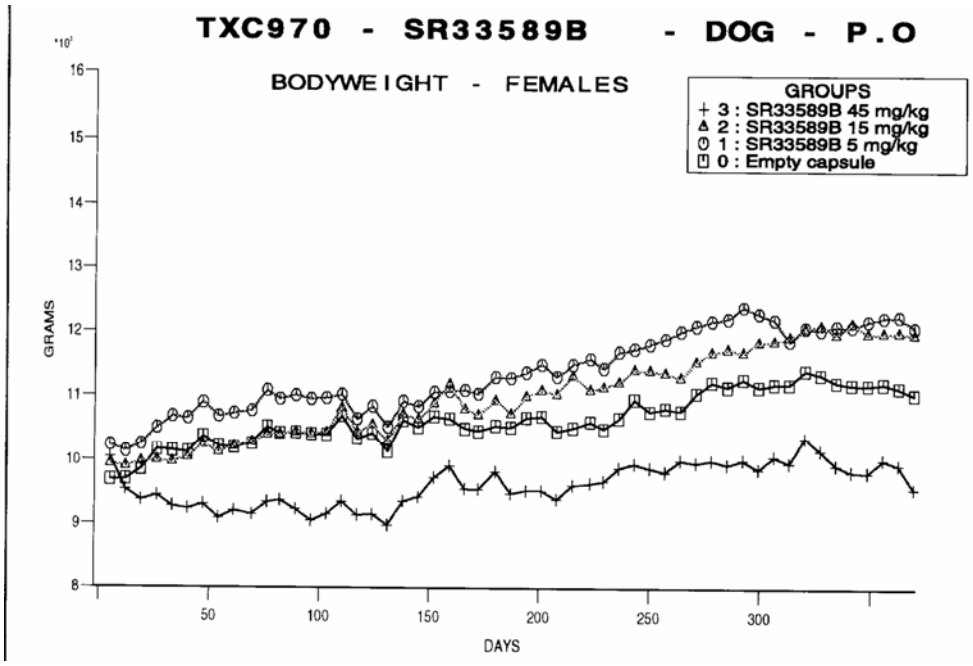
One male from the LD group was euthanized prematurely (day 202) due to declining condition referable to a bacterial enteric infection.

Ophthalmoscopy: no findings were reported

Body Weight

MD and HD males and females gained less than did the controls and LD animals.





There were no apparent effects on food intake.

ECG: There was a slight decrease in HR in the HD groups of both sexes that persisted from 93 days (earliest determination) to the end of the study. There was a great deal of variability in the T wave amplitude data making it difficult to discern any effects. QT and QTc prolongation (Bazett's formula) was seen in both sexes. The data from just day 364 is given as an example. PR prolongation (NS) was also seen in both sexes.

Males				
	Dose mg/kg			
	0	5	15	45
QT (0.01s) Day 364	18.6±0.49	18.8±0.93	19.3±1.17	23.2±0.36**
QTc Day 364	7.71±0.247	7.77±0.281	8.07±0.359	8.67±0.147**
Females				
QT (0.01s) Day 364	19.8±0.20	19.0±0.98	19.3±0.72	25.0±0.98**
QTc Day 364	8.20±0.197	7.74±0.175	7.88±0.291	8.96±0.252

Hematology and Clinical Chemistry

No consistent results in the hematology data as presented. The lymphocyte colony data did not show consistent trends. The lymphoblastic transformation tests also showed a great deal of variability. There may have been a decrease in transformation with increased concentration.

The clinical chemistry data did not indicate any signals not already seen in other studies. Many findings were also minimal and of questionable, if any, significance. Urea in HD males was increased at days 90 and 181 and within the range of the other groups by day 365. Urea was significantly increased in HD females day 365. Total cholesterol was reduced non-significantly in HD females only, at day 365. Phospholipids were decreased at this point also (NS). Total bilirubin was increased in all treated females on day 365 as shown on the next page. However, there is no corroborative data in the hematology so the meaning is unclear. Creatinine which in previous studies has shown relatively consistent increases in this study showed only minor increases in HDm (day 365) and moderate increases in HDf at the same time point.

Blood biochemistry : Total bilirubin (mcM) (Cont'd)

	Days			
FEMALE	-7	90	181	365
Levene	>10% NS	>10% NS	>10% NS	0.3% **
1-ANOVA	>10% NS	>10% NS	>10% NS	---
Group 0 : EMPTY CAPSULE				
n = 5				
mean	2.2	1.6	2.0	1.9
SEM	0.19	0.08	0.07	0.14
Group 1 : SR 33589B 5 mg/kg				
n = 5				
mean	2.5	1.9	2.2	2.6
SEM	0.29	0.15	0.19	0.42
Test/group 0				>10% NS
Group 2 : SR 33589B 15 mg/kg				
n = 5				
mean	2.4	1.4	2.0	2.3
SEM	0.30	0.13	0.20	0.18
Test/group 0				>10% NS
Group 3 : SR 33589B 45 mg/kg				
n = 5				
mean	2.4	1.7	1.7	2.7
SEM	0.24	0.24	0.20	0.21
Test/group 0				1.0% **

Serum sodium showed consistent decreases in both sexes on days 181 and 365 at MD and HD. Day 365 values are shown below by way of example. Serum potassium was decreased in the drug-treated females. Inorganic phosphate showed variability but appeared to be decreased in the HD females. Blood urea was increased in the HD group of both sexes from day 90 to the end of the study. The LD and MD groups of males showed increased urea day 365. In females, ALT was mildly increased in the HD group at all three determination times but probably of no biological significance. IgG and IgM concentrations showed variability also. No consistent patterns were discernible. T3 showed consistent decreases in males and slightly less consistent decreases in females. The ratio of T3/T4 was more consistently affected (decreased) in the female dogs than the males.

Summary of Clinical Chemistry Effects (Mean ±SEM)

Males				
	Dose mg/kg			
	0	5	15	45
Sodium mM day 365	146±0.32	144.8±0.48	141.4±0.68***	141.6±1.08*
K mM day 365	4.32±0.092	4.28±0.075	4.12±0.066	4.04±0.087
IgG mg/l day 181	4140±90	3955±112	4018±126	8100±653**
IgG mg/l day 365	11822±2994	10418±3014	8298±2470	5612±322
IgM mg/l day 181	1165±156	805±117	840±80	884±119
T3 nM day 365	1.34±0.062	1.35±0.070	1.02±0.075	0.72±0.055***
Females				
Triglycerides mM day 365	0.40±0.062	0.51±0.065	0.38±0.03	0.23±0.022
Choles mM Day 365	3.41±0.413	3.57±0.464	3.97±0.544	2.52±0.125
Sodium mM Day 365	145.0±0.32	143.4±0.75	142.8±0.86	142.8±0.37
K mM day 365	4.16±0.087	4.12±0.124	4.06±0.147	3.76±0.081
IgG mg/l day 181	4342±184	4112±85	4744±666	6392±1614
IgG mg/l day 365	5726±993	6100±1073	5400±1678	4344±245
IgM mg/l day 181	1303±258	885±117	1090±246	1119±260
T3 nM day 365	1.32±0.057	1.12±0.139	1.11±0.086	0.74±0.028***

* 1.0%<p≤5.0%; **0.1%<p≤1.0%; ***p≤0.1%

No apparent effects on the lymphocyte subpopulation percents were noted on either day 181 or day 365. There was a slight decrease in the absolute amount of lymphocytes day 181 in the HD groups. This change was not apparent day 365 and may have been normal variation. It was apparent that the variability inherent in the lymphoblastic transformation analysis may generate data in samples from pre-treatment animals that simulate a dose-response. It is difficult to say that there is any specific drug effect. However, the data generated with PHA and PWM suggests a decrease in transformation day 359 in males. Of note is the decrease in the variance of the drug-treated samples.

Lymphoblastic transformation test: PHA (cpm) day 359 in males

Dose group mg/kg	Without mitogen	Conc. 1	Conc. 2	Conc. 3
Empty capsule	274±54	12834±3308	14479±4869	15634±6456
5	266±16	12077±2375	14071±2897	15614±3172
15	197±13	7793±1700	10161±2810	9963±2887
45	296±44	8001±1936	9340±2289	9858±3397
PWM: day 184				
Empty capsule	239±36	6322±2093	6985±2065	9670±2600
5	217±32	7541±1465	7757±1702	10316±2317
15	259±37	5377±680	5258±700	7648±1100
45	294±44	5263±728	5663±614	8109±492
PWM: day 359				
Empty capsule	330±50	6468±2747	6203±2250	9010±3722
5	363±43	7514±1400	9113±2368	11573±2641
15	244±40	4417±990	4891±1122	6904±1331
45	313±22	4294±1247	4686±1099	6638±1312

Lymphoblastic transformation test: PHA (cpm) day 359 in females

Dose group mg/kg	Without mitogen	Conc. 1	Conc. 2	Conc. 3
Empty capsule	385±121	10012±1739	13935±4020	14612±5150
5	213±12	6958±1022	8120±1356	8492±2154
15	300±46	7734±1879	10607±2978	13218±4518
45	268±31	8814±1255	12813±1592	11865±1874
PWM: day 184				
Empty capsule	159±18	4655±1448	5266±1671	7526±2329
45	272±31	3736±463	3781±490	5054±1052
PWM: day 359				
Empty capsule	305±39	6826±2544	7164±2377	9809±3359
45	242±24	5070±945	4815±661	6398±613

In the urinalysis, creatinine excretion was decreased in HD males days 177(42% vs controls) and 363(54% compared to controls, ($1.0\% < p \leq 5.0\%$). Creatinine excretion was decreased in all groups of drug-treated females without a dose-related pattern.

Urine concentration of creatinine Day 363

Females	0	5 mg/kg	15 mg/kg	45 mg/kg
Creatinine mM	16.1±2.41	7.8±1.59	8.1±1.68	10.8±2.91

A clear dose-related pattern was not seen, but urinary sodium and potassium were decreased in the HD males days 86, 177 and 363. Urinary sodium was increased in the HD females days 86 and 177 and slightly decreased day 363. Urinary potassium was slightly decreased in the HD females day 177 and decreased in a non-dose-related manner all the drug-treated groups.

Bacteria in the urine cytology was variable and showed no consistent pattern. The significance if any is unclear as handling and storage may affect these results.

Organ weights

Weight effects were seen in the same organs as identified in other studies.

Summary of organ weight effects

males				
	Dose mg/kg			
	0	5	15	45
Heart g/kg	7.62±0.272	7.57±0.289	8.51±0.640	10.39±0.282 ***
Lungs g/kg	7.05±0.584	6.30±0.486	8.04±0.767	8.11±0.387
Brain g/kg	5.72±0.485	5.91±0.387	6.22±0.359	7.6±0.449*
Thyroid mg/kg	72±12.1	70±3.7	73±5.5	86±8.6
Testes g/kg	1.210±0.186	1.303±0.119	1.492±0.103	1.511±0.100
Females				
Heart g/kg	8.15±0.460	7.05±0.512	7.67±0.529	9.63±0.384
Lungs g/kg	6.99±0.244	6.32±0.370	6.76±0.716	7.20±0.436
Brain g/kg	6.44±0.349	6.35±0.442	6.46 ±0.428	7.69±0.413
Thyroid mg/kg	87±4.7	81±8.2	78±6.0	97±13.3

	Gr. 0		Gr. 1		Gr. 2		Gr. 3	
	M	F	M	F	M	F	M	F
Kidneys (cont'd)								
Slight congestion							31	
Very slight to moderate, focal or multifocal chronic inflammatory cell infiltration		10"			23" 29'		31"	
					24" 28"*		32'	
							33"	
	* fibrosing " cortical ' medullary							
Few papillary mineralizations	1	7	12	16	21	27	32	36
	3	9	13	17	22	28	35	37
	5		14s	18	23	29		38
				19		30		39

Lungs (cont'd)

Slight or moderate, focal, zonal or multizonal emphysema	:	2"	6":	11"	17":	21	26":	33"	36":
	:	3"	7 :	13	18":	22	27":	34"	37":
	:	4"	8":	15"	19 :	23	29":		38 :
	:	5	9 :		20":		:		40 :
	"subpleural								
A few areas of atelectasia									
	:		:		:		27 :		:
Foci or infiltration by foamy cells									
	:	2	6 :		18 :	21	26":	32"	36 :
	:	5	7 :		:		:		38 :
	:		:		:		:		39 :
	:		:		:		:		40 :
	"subpleural								

	Gr. 0		Gr. 1		Gr. 2		Gr. 3	
	M	F	M	F	M	F	M	F

Female genital tract

Pseudocyesis								
	:	8 :		16 :		:		36 :
	:		:	17 :		:		:
	:		:	20 :		:		:

Prostate

Chronic and/or fibrous inflammatory cell area(s) in the prostate								
	:		:	11	:	22	:	31
	:		:	15	:	24	:	34
	:		:		:	25	:	

Toxicokinetics

Increases in AUC exposure to parent drug were greater than proportional all three sampling days. Exposure did not change over the course of the study, suggesting that there was no apparent saturation phenomenon, no induction of metabolism and no accumulation of compound.

Toxicokinetic Summary

Dose Mg/kg/day	Cmax (mg/l)		AUC_{0-24h} (mg.h/l)	
	males	females	males	females
Day 30				
5	0.007±0.007	0.041±0.011	Nc	0.147±0.035
15	0.331±0.019	0.244±0.091	3.140±0.126	2.053±1.350
45	1.465±0.360	2.242±0.458	20.863±4.229	38.026±8.095
Day 188				
5	0.000±0.000	0.000±0.000	Nc	Nc
15	0.202±0.029	0.204±0.158	2.423±0.161	1.696±1.280
45	1.540±0.147	1.653±0.365	21.232±3.515	25.274±5.611
Day 366				
5	0.015±0.008	0.045±0.010	Nc	0.139(nc)
15	0.254±0.025	0.181±0.107	2.687±0.187	2.359±0.811
45	1.437±0.206	1.289±0.272	22.407±3.780	22.007±3.716

Exposure to the major metabolite was determined for the HD group only. There was no apparent difference (no accumulation or induced metabolism) in exposure at the 3 different sampling times.

Summary of Toxicokinetics for SR35021

Sampling day	Cmax (mg/l)		AUC0-24(mg.h/l)	
	males	females	males	females
30	6.086±0.019	0.175±0.034	1.006±0.274	2.549±0.557
188	0.092±0.009	0.161±0.033	1.281±0.186	2.524±0.219
366	0.080(NC)	0.137±0.013	0.896(NC)	2.152±0.009

Study title: Two-week intravenous toxicity study in the dog

Key study findings: Consistent with other studies, first degree AV block was seen in a HD female. QT prolongation was also seen. Control animals did not show any local signs, therefore, the sponsor ascribed poor tolerance to the infusion process rather than the drug. The sponsor reported that there was slight to marked edema and induration. The dose 1mg/kg was associated with moderate intolerance and 2.5 mg/kg was associated with marked intolerance. Local intolerance made it difficult to impossible to administer drug, resulting in an increased number of injection sites. This resulted in 1/3 animals in the HD group being euthanized and 1 MD male receiving only half of the final dose. Venous thrombosis and phlebitis were also reported. Serum electrolytes and excreted electrolytes, primarily sodium, showed some alterations with drug treatment. The primary organ weight changes were in the liver and the reproductive tracts. The sponsor describes the changes in the female reproductive tract as being physiological. However, this is not clear from the data presented.

Study no.: TSA0885
Conducting laboratory and location: Sanofi Recherche, Montpellier Cedex, France
Date of study initiation: May 26, 1993
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589B, lot 92.01, purity
Formulation: PEG400, acetic acid, sodium acetate buffer
Vehicle: 5% glucose solution
Injection given over 30seconds

Methods Beagles, approximately 1 year old, 3/sex/group were given intravenous doses of 0, 1, 2.5, 6 mg/kg/day once daily for 13 to 19 days. The last treatment day was the day before necropsy.

Clinical signs: 2x/day
ECG: days-7, 13, 2-4 hours after dosing. Leads I, II, III, aVR, aVL, aVF. QTc was calculated by Bazett's formula.

Ophthalmoscopy: days -1, 10
Body weight: pre treatment and weekly
Food intake: daily per animal and group means calculated weekly
Hematology and clinical chemistry: days-9, 14
Urinalysis: performed on overnight samples collected by unspecified means days-1, 10
Toxicokinetics: day 15 collected blood at 3 and 24 hours from:

Low dose: 6 animals
Mid dose: 4 animals
High dose: 3animals

Necropsy: performed from day 16-20. gross observations made, tissues collected for light microscopy, EM samples unclear from chart

Results
P values included in the tables have the following statistical significance:

* $1.0\% < p \leq 5.0\%$
** $0.1\% < p \geq 1.0\%$
*** $p \leq 0.1\%$

The sponsor's summary of observations is reproduced here.

Observations/group-dose mg/kg/day	0(0)	1(1)	2(2.5)	3(6)
# of injection sites	16	25	36	32
# of animals in which injection could not be done	0	0	1(day15, ½ the dose)	1(day 14, euthanized)
# of animals w/lesions	0	3	5	5
# of sites with lesions	0	5	16	15
#of observations/# of days x# of animals				
total	0/114	18/99	87/92	92/96
slight edema	0	16	31	48
marked edema	0	0	0	5
slight induration	0	2	53	35
limb or area(*)	0	0	3	4

* limb or area = increased limb or area (neck, thigh) volume with a firm consistency

Local intolerance to the vehicle was distinguished from a drug effect by the addition of thrombosis in the drug-treated groups.

Sponsor's summary of macroscopic injection site findings

Dose mg/kg/day	0	1	2.5	6
# of animals	6	6	6	6
# of sites sampled	13	27	46	40
Edema: slight			1	
Moderate		1	3	5
Marked				4
Whitish induration in the subcutaneous tissues				
Slight	8	7	7	2
Moderate	1	9	13	10
Marked		2	4	9
Hematoma: small – moderate	3	9	24	19
Large				3

Summary of injection site microscopy (Sponsor's table)

	Dose mg/kg			
	0	1	2.5	6
# of animals	6	6	6	6
# of sites examined	13	27	46	40
Vascular changes				
Principal vein thrombosis	-	9	16	14
Collateral vein thrombosis	-	3	8	6
Phlebitis	-	5	11	16
Perivascular changes				
Infiltration of hemodynamic origin				
Very slight or slight	5	3	11	1
Moderate or marked	2	2	5	13
Very slight to marked inflammatory cell infiltration	6	10	17	13
Hypodermic changes or changes of the fatty tissue adjacent to the vein				
Infiltration of hemodynamic origin				
Very slight to slight				
Moderate to marked	3	6	7	6
Very slight to marked inflammatory cell infiltration	3	8	15	19
	4	15	24	20

The sponsor noted on page 40 that thrombosis and phlebitis were seen only in animals receiving SR33589B. The hemodynamic and inflammatory perivascular changes already present with vehicle were exacerbated by the addition of drug.

Diarrhea was reported in 1 male from the 6 mg/kg group. Male #21 was euthanized on day 14 as the intravenous injection could not be performed on him that day (reason unspecified). Drug-treated animals ate less and gained less weight than did the control groups.

ECG: 1st degree AV block was seen in 1/3 HD f. This is consistent with other studies. PR duration was increased in both sexes at the HD: male control 10.2±0.71 vs 12.0±0.57 (0.01s) for the HD males and 10.0±0.71 female controls vs 12.0±1.37 for HD females. Not statistically significant for either sex.

QT interval was increased for all drug-treated groups but not in a dose-related fashion. QTc was increased for all male drug-treated groups and the HD females.

Ophthalmoscopy: no treatment-related changes were reported.

Hematology and Clinical Chemistry

PCV and hemoglobin showed slight apparently dose-related decreases in both sexes. Day 14 hematology showed an increased number and percent of neutrophils and slightly increased monocytes in the drug-treated animals. This is consistent with the tissue irritation and damage from the injections. Fibrinogen was increased in all groups compared to baseline. The levels in the HD groups were elevated compared to the control groups. This is again consistent with an inflammatory process and a difference in levels of inflammation between the vehicle and the drug.

Day 14, serum creatinine decreased somewhat in the drug-treated groups in a relatively dose-related way. Globulins increased slightly in the MD and HD females. Serum sodium decreased in a dose-related manner in the males and non-dose-related in the females. Serum potassium was lower in drug-treated animals than control but without a discernible pattern.

Summary of serum biochemistry

Males				
	Dose mg/kg			
	0	1	2.5	6
Sodium mM	150.3±1.20	148.7±0.33	145.7±0.67*	143.5±2.50
K mM	4.47±0.088	4.07±0.033	4.10±0.058	4.10±0.200
Females				
Sodium mM	150.0±0.58	147.7±1.20	148.0±0.58	146.7±1.20
K mM	4.17±0.067	4.30±0.000	4.00±0.115	4.17±0.120

There were mild differences in electrolyte excretion in the drug-treated groups.

Summary of Urinalysis Results

Males				
	Dose mg/kg			
	0	1	2.5	6
Na mM	84±18.8	66±19.1	77±5.7	71±11.3
K mM	31.9±0.99	22.9±6.39	19.8±1.62	26.2±8.30
Females				
Na mM	121±63.7	45±6.1	71±0.6	60±6.6
K mM	18.3±2.11	28.1±10.28	22.7±2.71	30.7±6.45

Liver weight was increased primarily at the HD. Absolute testes weight showed a dose-related decrease. Absolute and normalized uterine weight was increased as was ovarian weight.

Summary of Organ Weight Effects

Males				
	Dose mg/kg			
	0	1	2.5	6
Liver (g) Liver g/kg	295±22 26±1.5	277±23 27±3.0	313±18 30±1.0	328±30 30±4.1
Testes (g)	17±1.4	16±1.2	15±1.3	12±1.0
Testes (g/kg)	1.49±0.14	1.55±0.15	1.41±0.11	1.14±0.08
Females				
Liver (g) Liver (g/kg)	294±11 27±0.5	275±9 28±1.0	290±20 28±0.5	330±37 36±4.4
Adrenals mg/kg	112±4.0	112±3.0	109±5.4	160±30
Eyes (g/kg)	0.996±0.055	1.127±0.016	1.077±0.095	1.181±0.030
Brain (g/kg)	6.76±0.54	7.71±0.12	8.08±1.17	8.50±0.33
Pituitary (mg/kg)	5.6±0.55	6.6±0.49	6.6±0.53	6.3±1.28
Uterus (g) Uterus g/kg	5.2±2.4 0.46±0.19	9.4±5.2 0.971±0.54	13.0±5.3 1.29±0.53	12.0±5.5 1.26±0.56
Ovaries (mg) Ovaries (mg/kg)	729±110 66±12	1106±357 114±37	1250±350 121±32	1483±556 156±54

Toxicokinetics: SR35021 was not detected in the plasma of the animals sampled in the three treated groups. Three hours after dosing SR33589 was present in quantifiable amounts in the plasma of the sampled animals with the exception of 1 LD male. By 24 hours after dosing SR33589 was found only in 1 HD female. Neither Cmax nor AUC_{0-24} were assessed.

Study title: Preliminary toxicity study by intravenous infusion to beagle dogs

Key study findings: As a dose-ranging study for the longer intravenous studies, first degree block was again reported as was thrombosis and phlebitis. I could not find a numerical summary of thrombi and vascular changes. One incident of second degree heart block was also reported.

Study no.: DDO0549
Conducting laboratory and location: Pharmaco L.S.R., LTD, Eye, Suffolk, England
Date of study initiation: September 14, 1994
GLP compliance:

QA report: yes ( ) no ( )
Drug, lot #, and % purity: SR33589B lot L119E, vehicle of mannitol

Methods Eight month old Beagles, 1/sex/group, were given intravenous doses of 5, 10 or 20 mg/kg day. The duration varied with the treatment. The study design is summarized in the sponsor's table reproduced below:

Sponsor's Summary of Study Design

Group 1	Saline solution	20 ml/kg	D1- D5	1 hr infusion
Group2	vehicle	20 ml/kg	D1- D2 D3- D5	1 hr infusion 2 hr infusion (dilution)*
Group3	5 mg/kg/day	D1-D2 D3- D4	5 ml/kg 10 ml/kg	1 hr infusion 2 hr infusion (dilution)*
Group 4 Male only	10 mg/kg/day	D1-D2 D3-D5	10 ml/kg 20 ml/kg	1 hr infusion 2 hr infusion(dilution)*
Group 5	20 mg/kg/day 10 mg/kg/day	D1 D3	20 ml/kg 20 ml/kg	1 hr infusion 2 hr infusion (dilution)*
Group 6	10 mg/kg/day	D8-D21	10 ml/kg	1 hr infusion
	Animals 4M and 1F reassigned as radiographs showed that their cannula were lying beyond the jugular vein in the vena cava.			
Treatment duration	5 days: groups 1-5 (Day 1-Day 5) 14 days: group 6 (Day 8 to day 21)			

*dilution= dilution in 5% dextrose

Animals were monitored for signs, body weight, food intake, ECG, blood pressure, hematology, clinical chemistry

Statistical analysis: p values included in the tables are associated with the following statistical significance:

*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Results

Two males and 1 female showed no treatment-related changes.
1 male, 1female: severe hypotensive episode on day 1
Those two animals who experienced severe hypotension and 1 other male and 2 other females were euthanized early due to severe reaction such as: decreased activity, tremors, rapid heart beat, pyrexia, swelling around the surgery site and limited use of the forelimb.

1male and 1 female, both from group 6, showed a first degree AV block immediately after dosing. The male also showed second degree heart block prior to dosing on day 14. The animals euthanized prematurely showed elevated fibrinogen and WBC (neutrophil counts), lower platelet counts, shorter thrombin time and slightly shorter prothrombin

times, consistent with an inflammatory and infectious process. The early decedents also showed higher alkaline phosphatase levels, higher triglycerides and cholesterol levels, low calcium and potassium levels. Albumin was decreased and globulin levels were somewhat elevated.

Four out of 5 animals showed a slight anemia.

The sponsor makes an interesting point in the textual summary: "Apart from the hypotensive episode noted in animals that received 20 mg/kg/day, little relationship to dosage was evident in the extent of toxicity noted in these animals and dilution of test material did not have any noticeable activity in alleviating the symptoms [sic] seen [page 45]." I could not find a numerical summary of thrombi and vascular changes.

Study title: Toxicity study by intravenous infusion to Beagle dogs for four weeks

Key study findings:

Study no.: TSA0962

Conducting laboratory and location: Pharmaco LSR Ltd, Eye, Suffolk, England
Date of study initiation: December 6, 1994
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589 batch 92.01, vehicle of mannitol

Methods Beagles, 3/sex/group received SR33589 for one hour per day by intravenous infusion at dosages of 0, 1, 2 or 4 mg/kg/day for a 4 week treatment period. Catheters were installed in the jugular veins. Saline and vehicle control groups were included. Animals were monitored for signs, bodyweight, food consumption, ophthalmoscopy, electrocardiography, hematology, clinical chemistry and urinalysis. PK samples were collected during the final week of treatment before and immediately after dosing and 1,3 and 9 hours after the end of infusion. Necropsy examination included gross observations, organ weights and histopathology. Liver and kidney samples were collected from all animals for EM.

Statistical analysis: p values included in the tables are associated with the following statistical significance:

*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Results

No clinical signs or unscheduled mortality were reported.

There were no apparent effects on body weight or food consumption.

Hematology and Clinical Chemistry

A slight anemia was apparent, characterized by decreased PCV, Hb and RBC count. Total WBC count was increased in the drug-treated males, primarily due to a neutrophilia and secondarily to lymphocytosis and monocytosis, suggesting some chronicity to the infectious/inflammatory stimulus. Females showed an increased neutrophil count and a mild increase in monocytes was apparent in the HD group.

There were no consistent or dose-related ECG findings that occured in both sexes. Females showed increased PR and QRS intervals. QT and QTc were apparently unaffected. Amplitudes of Q,R, and S were altered compared to control. The ratio of R:T was also affected.

Masses were seen at necropsy in all groups of animals at the infusion sites. Some distant changes in the hearts (ventricles) and other veins were reported also but without an apparent dose-response relationship. The masses were described as thickened endocardial and intimal areas after microscopic examination. The thickened areas were cellular and sometimes fibrocellular with cellular elements either fibrocytes or myocytes. Leucocytic cell infiltration was seen in the majority of these cases. Focal areas of necrosis were seen. Thus, pathological reaction at the infusion sites and associated vasculature were seen in all groups including both control groups. A dose-response relationship to the vascular irritation was not found.

Possibly due to the small group size, treatment-related changes could not be discerned in the clinical chemistry data, urinalysis, organ weights and pathology.

Study title: 4-week intravenous infusion toxicity study in the dog: toxicokinetic data

Key study findings: SR35021 was not detected. SR33589 was detected in both sexes at all doses. There were no obvious differences between the sexes. The results were close to dose-proportional.

Study no.: TSA0962

Conducting laboratory and location: TK portion conducted at Sanofi Recherce, Montpellier Cedex, France
Date of study initiation: in life December 1994; analysis February 1995
GLP compliance: yes
QA report: yes ( ) no ( )
Drug, lot #, and % purity: SR33589B batch 92-01

Methods Determination of SR33589 and SR35021 in plasma by HPLC with UV detection. LOQ: 0.020 mg/l LOD: 0.010 mg/l

Results SR35021 was not detected.

SR33589 pharmacokinetic results (mean±SD)

Dose mg/kg/day	Males		Females	
	C1h mg/l	AUC_{0-24}(mg.h/l)	C1h mg/l	AUC_{0-24}(mg.h/l)
1	0.087±0.019	0.287±0.038	0.067±0.019	0.167±0.051
2	0.246±0.059	0.605±0.085	0.190±0.057	0.571±0.057
4	0.420±0.128	1.303±0.139	0.422±0.135	1.400±0.305

Study title: Four-day intravenous dose-ranging study in the macaque

Key study findings: This was a dose-ranging study for a proposed longer IV dosing study in non-human primates. Consistent with other intravenous administration studies, poor local tolerability was seen at the injection sites, attributed primarily to the vehicle but, also consistent with other studies, exacerbated by the drug as shown by thrombosis. Dose-dependency for the thrombotic effects was reported as lacking. Drug-treated animals also showed evidence of anemia. ECG changes were seen at doses ≥4mg/kg consisting of 20-30%decreased heart rate (≥4mg/kg) and increased QT and PR intervals. First degree AV block was reported. A male treated with 16 mg/kg died after showing profoundly altered ECG parameters. A female given the same dose went into tonic seizures also accompanied by ECG changes. Recovery was reported to occur after 15 minutes. It was interesting to note that ECG changes were reported to occur at the lower doses 1 hour after IV administration, suggestive of a metabolite effect. ECG changes at the highest dose were reported to occur after the injection but be resolved 15 minutes after the injection.

Study no.: DDO0503
Conducting laboratory and location: Sanofi Recherche, Montpellier Cedex, France
Date of study initiation: January 18, 1993
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity: SR33589B batch DJ.07.51.5, vehicle of PEG400, acetic acid and acetate buffer.

Cynomologous monkeys were used.

Sponsor's summary of experimental design

group	animals		compound	Dosage mg/kg/day	Route
	number	first			
0	1M 1F	1 2	Vehicle	----------	IV
1	1M 1F	3 4	SR33589B	2	IV
2	1M 1F	5 6	SR33589B	4	IV
3	1M 1F	7 8	SR33589B	8	IV
4	1M 1F	9 10	SR33589B	16	IV

Animals were examined for signs, conscious ECG, body weight, food consumption, hematology, bone marrow, coagulation, clinical chemistry, toxicokinetics, gross pathology, organ weights, histopathology. Selected liver and kidney samples were analyzed by EM.

Statistical analysis: p values included in the tables are associated with the following statistical significance:

*significant difference with $1.0\% < p \leq 5.0\%$
**significant difference with $0.1\% < p \leq 1.0\%$
***significant difference with $p \leq 0.1\%$

Results:

Unscheduled mortality was seen. The male treated at 16 mg/kg/day died 5 minutes after the first dose (20 second injection). ECG changes before death included :

notched T-wave with a reversal in polarity
sino-atrial block with intermittent junctional rhythm
complete AV dissociation
ST segment depression
Junctional followed by ventricular escape rhythm

The female treated at 8 mg/kg was prostrate during 10 minutes after the injection on the first day of treatment. The female treated at 16 mg/kg day (5 minute injection) showed tonic convulsions during a few minutes at the end of the injection on the first day of treatment associated with ECG changes. The ECG changes seen in the female were:

Gradual decrease in heart rate with concurrent increase in PR interval
10 minutes later, Transient sino-atrial block with transient junctional rhythm, associated with ST segment depression
followed by sinus rhythm and bradycardia
Changes were reported to resolve by 15 minutes after the injection.

Changes on the second day:

No changes during the injection
After injection, HR decreased in association with a depressed T-wave
No other changes were listed
Changes were again reported to have resolved by 15 minutes after the injection

Other recorded and/or described ECG effects include:

Animals treated at 4 mg/kg and 8 mg/kg at 1 hour after treatment showed a 20-30% decrease in heart rate (with prolonged RR interval), first degree AVB (prolonged PR interval) and somewhat prolonged QT interval.

The male treated at 16 mg/kg/day didn't show any changes during the first administration. Immediately after the injection, the animal showed notched and reversed T-wave polarity associated with decreased heart rate and sino-atrial block with intermittent junctional rhythm. In a few minutes, complete atrio-ventricular dissociation was noted with ST segment depression, junctional followed by idioventricular escape rhythm and eventually death.

The female treated with 16 mg/kg didn't show any changes during the first administration. A few minutes later, the animal had a convulsion, during which, HR decreased while the PR interval increased. Ten minutes later, sino-atrial block with transient junctional rhythm was observed with ST segment depression and sinusoidal rhythm with bradycardia. No further modifications were noted from 15 minutes after the injection.

Local tolerability changes were reported for the last day of treatment. Diffuse redness was reported for the control animals, diffuse edema in the LD m and local edema in the LD f. Diffuse redness was reported for the 4 mg/kg f and diffuse edema for the 8 mg/kg m. At necropsy, poor tolerability was concluded, aggravated by thrombosis in those animals who had received SR33589B, with no dose related effect.

The drug produced degrees of anemia in all treated animals except the LD male. A leucocytosis and neutrophilia was also reported for males from ≥2 mg/kg and in the female treated with 16 mg/kg/day. There was also a slight increase in fibrinogen in the HD f. These changes are consistent with inflammation/infection and poor tolerability.

The following clinical chemistry changes were ascribed to drug treatment: slight decreases in glucose levels, moderate decreases in albumin, decreased potassium, chloride and calcium levels in the female treated at 16 mg/kg/day. Increased LDH activity was seen in all animals including controls. This could be related to irritant properties of the vehicle.

Histopathology of the injection sites showed thrombotic changes and subacute phlebitis. Perivascular changes included hemorrhagic or fibrino-hemorrhagic nature and subacute inflammation.

Sponsor's summary of microscopic changes related to the injection

	Dose mg/kg/day				
	0	2	4	8	16
Number of animals	2	2	2	2	2
Vascular changes					
Thrombosis	-	2	2	1	1
Subacute phlebitis	-	2	2	1	-
Perivascular changes					
Infiltration of hemodynamic nature	1	2	-	1	2
Inflammatory infiltration	-	2	2	2	1
Subcutaneous changes					
Infiltration of hemodynamic nature	2	2	2	2	1
Inflammatory infiltration	1	1	1	2	1

Study title: 2-week intravenous toxicity study in the macaque

Key study findings: Consistent with the other intravenous administration studies, there was a dose-related increase in tissue irritation (≥1 mg/kg) and damage with profound gross and microscopic changes of hemorrhage, inflammation and thrombosis as well as muscle necrosis. The sponsor noted in the text that they summarized only the "maximal changes". Not presented in the summary table but mentioned in the text were changes in veins of the axilla, cases of vascular rupture, segmental venous necrosis and muscular and dermal hemorrhagic infiltration, local opportunistic infections, a few cases of widespread necrosis of the crural muscle, with inflammatory, septic, edematous and hemorrhagic changes sometimes associated. Performing injections became technically difficult after 4 or 5 days for all dose groups. A male HD monkey and a female HD monkey died prior to scheduled termination. The findings in the female included multi-focal acute hemorrhagic endomyocarditis in the left ventricle. While the cause of the endocarditis was undetermined, there are plausible causes not directly related to the drug. E.g., infectious. The male showed increased serum glucose, ALT, AST and LDH and increased urinary potassium excretion. Both animals showed proteinuria. The hematology in all drug-treated animals indicated primarily an inflammatory response. Mild, regenerative anemia was seen in females at doses ≥2.5 mg/kg and at 6 mg/kg in males. Decreased serum albumin, triglyceride levels and slightly increased liver weight suggest the liver as one of the target organs. Both absolute and normalized uterine and ovarian weight were decreased in what appears to be a dose-related manner. Given the other reported changes, the changes in these reproductive organs may have been secondary to chronic sympathetic discharge.

Study no.: TSA0884
Conducting laboratory and location: Sanofi Recherche, Montpellier Cedex, France
Date of study initiation: May 18, 1993
GLP compliance: statement included
QA report: yes (x) no ( )
Drug, lot #, and % purity:SR33589B batch 92.01 vehicle of PEG400, acetic acid

The animals were treated once daily for 14 -16 days. Control animals received the vehicle at a similar volume as the high dose animals. Solutions were administered as a bolus injection over 10-20 seconds. The sponsor's summary of design is reproduced below.

Sponsor's summary of experimental design

group	#of animals	First animal number	compound	Dose mg/kg/day	Route of administration
0	3M 3F	1 4	Vehicle	0	IV
1	3M 3F	7 10	SR33589**B**	1	IV
2	3M	13	SR33589**B**	2.5	IV

	3F	16			
3	3M 3F	19 22	SR33589**B**	6	IV

Observations included clinical signs, body weight and food consumption, ECG, ophthalmoscopy,
Hematology, clinical chemistry, urinalysis, gross necropsy, organ weights,
Statistical significance:
* 1.0%<p≤5.0%; **0.1%<p≤1.0%;***p≤0.1%

Results

The following changes were observed at 6 mg/kg: pallor of mucous membranes from day 9 onwards, weakness in 1m and 1 f, abnormal gait in 2m and 1 f, prostration in 1f. One male and 1 female from this group were found dead days 14 and 12 respectively. The male showed weakness the day before. Acute, multifocal endomyocarditis was found in the left ventricle of the female.

At all doses, the injections became technically difficult after 4 or 5 days. The site of the injections needed to be changed several times during the course of the study. In all drug-treated groups, redness was observed at the injection site as well as induration. From ≥2.5 mg/kg, scabs, oozing or edema were noted more in drug-treated than in control animals. A few animals presented with severe and extensive lesions. Limbs were increased in volume and in some cases more than 1 limb was involved. At the highest dose tested, 3 cases of necrotic lesions were reported in hindlimbs. The sponsor's summary of observations on this point is reproduced below.

Macroscopic observations

	Dose mg/kg			
	0	1	2.5	6
# of animals	6	6	6	6
#of injection sites	12	36	35	38
wound				3
edema	0	3	4	7
hematoma	7	15	12	9
induration	1	15	13	16
abscess	1		2	
Microscopic observations				
Necrosis (perivascular and perimuscular)	0	1	3	8
Exulceration focal	0	1	1	0
Ulceration: focal –very widespread	0	1	1	3

Injection sites and associated organs and tissues were described as having organized or recanalized thrombi. Adjacent muscles shoed slight to marked acute fibrous inflammatory cell infiltration and necrotic tissue. Limbs were also reported to show ulceration.

At doses ≥ 2.5 mg/kg: Health was deteriorating as evidenced by lymphoid atrophy and involution of thymus, spleen and lymph nodes and slight-moderate cortical hyperplasia of the adrenals. Other findings were decreased hepatic glycogen, slight-moderate cortical tubular swellings in the kidneys, serous de-differentiation of the pancreas (1/6 MD and 3/6 HD) and slight atrophy of the intestinal mucosa (1/6 HD, no further details).

ECG: HR decreased in the HD groups of both sexes (16% in males , 13% in females compared to baseline). PR duration was increased in males but showed inconsistent, non-dose-related increases in females. T amplitude was decreased by approximately 50% in HD males and from 36-46% in females.

Body weight: Drug-treated animals either gained less than the controls or actually lost weight. A dose-related pattern was not apparent. No NOAEL was defined.

Hematology and Clinical Chemistry

Hematology showed that the drug-treated animals had a regenerative anemia with a pronounced increase in reticulocyte count. No NOAEL was seen. Mild leucocytosis was seen in both sexes, primarily due to neutrophilia. Platelet counts were increased in the MD and HD groups of both sexes also. APTT and thrombin time were increased in males.

Albumin showed a dose-related decrease in males and was decreased in MD and HD males. Triglycerides were decreased in both sexes of drug-treated animals. Bilirubin was increased in both sexes of drug-treated animals. Total cholesterol was decreased in males.

ALT and AST were increased in the HD of both sexes. LDH activity was increased in the MD and HD of both sexes. Ca and K were decreased in the HD of both sexes.

Organ Weights

Both absolute and relative uterine and ovarian weights were decreased in the MD and HD groups. There is no histopathology or data regarding the cyclicity of these animals, making the weight findings difficult to interpret. In males, the absolute and relative liver, adrenal and spleen weight was increased in the HD. Absolute and relative kidney weight was increased in all drug-treated groups.

In females, absolute and relative kidney weight was slightly increased in the MD and HD groups. Absolute adrenal weight was increased in all drug-treated groups without a dose-response.

Summary of organ weight changes

	Dose mg/kg/day			
	vehicle	1	2.5	6
Males				
Adrenal mg/kg	246±3.5	245±27.5	269±19.5	407±59.1
Eyes g	5.87±6.20	6.20±0.161	6.11±0.145	6.33±0.042
Brain g	62.55±1.965	68.72±1.943	61.82±1.355	74.24±2.658*
Brain mg/kg	25.16±1.156	25.93±1.610	24.68±1.854	29.71±0.244
Females				
Adrenal mg/kg	221±9.1	285±20.7	394±59.2	335±34.5
Uterus (g)	3.96±1.37	4.22±0.98	2.22±0.17	1.57±0.16
Uterus g/kg	1.49±0.48	1.77±0.41	1.08±0.09	0.69±0.04
Ovaries (mg)	252±85.5	192±38.2	113±10.7	123±3.0
Ovaries mg/kg	94±24.0	81±16.3	55±5.8	55±4.2
Brain g/kg	23.72±1.37	27.07±2.33	29.23±0.65	28.43±2.22

Histopathology Findings

The histopathology results provided were primarily concerned with the injection sites and findings related to the irritancy potential of the vehicle/drug combination.

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/s/

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