

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
NDA 22-220

PHARMACOLOGY REVIEW(S)



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22-220
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 8/15/2007
DRUG NAME: TRIVARIS™ (Triamcinolone acetonide injectable suspension, USP) 8%
INDICATION: Sympathetic ophthalmia, temporal arteritis, uveitis, and ocular
inflammatory conditions unresponsive to topical corticosteroids
SPONSOR: Allergan, Inc., 2525 Dupont Drive, P.O. Box 19534, Irvine, CA
Tel: 800-347-4500; Fax: 714-246-4272
REVIEW DIVISION: Division of Anti-Infective and Ophthalmic Products
PHARM/TOX REVIEWER: Zhou Chen, MD, PhD
PHARM/TOX SUPERVISOR: Wendelyn Schmidt, PhD (acting pharmacology/toxicology team leader)
DIVISION DIRECTOR: Wiley Chambers, MD (acting division director)
PROJECT MANAGER: Raphael Rodriguez

Date of review submission to Division File System (DFS): March 26, 2008

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This application is approvable from a nonclinical perspective with some modifications of labeling as revised in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section.

B. Recommendation for nonclinical studies

No recommendation is necessary.

C. Recommendations on labeling

Modifications of labeling in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section (see below).

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

No adequate studies have been conducted in animals to determine whether corticosteroids have a potential for carcinogenesis

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Triamcinolone acetonide was not mutagenic or clastogenic in the Ames bacterial reversion test and chromosomal aberration assay in Chinese hamster ovary (CHO) cells. Positive results were noted in the in vivo micronucleus test with triamcinolone acetonide in mice.

Steroids may increase or decrease motility and number of spermatozoa in some patients.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Triamcinolone acetonide is a synthetic corticosteroid with anti-inflammatory activity. Triamcinolone acetonide is currently marketed under the trade name Kenalog[®]-40. The current NDA is a 505(b)(2) submission. The application relies on published literature for safety and effectiveness and on the Agency's previous finding of safety and effectiveness for triamcinolone acetonide.

In ocular PK studies in NZW rabbits, following an ITV administration, high exposures to triamcinolone acetonide (TA) were seen in the vitreous humor and retina. The 4% triamcinolone acetonide prototype formulation generated similar intraocular triamcinolone acetonide concentrations compared to Kenalog[®]-40 at the same dose. The systemic exposure to TA following ITV injection was very low.

In two ocular toxicity studies with triamcinolone acetonide, reversible ocular findings, including congestion, swelling and/or ocular discharge and tearing, were seen in both drug- and vehicle-treated animals, suggesting that the findings might be related to the injection procedure. From the two ocular

toxicity studies, it was concluded that there was no toxicologically significant, drug-related toxicity with triamcinolone acetonide by intravitreal injection.

Triamcinolone acetonide was positive in the *in vivo* micronucleus test with triamcinolone acetonide in mice.

B. Pharmacologic activity

Triamcinolone acetonide is a synthetic corticosteroid with anti-inflammatory activity. Synthetic corticosteroids such as triamcinolone are primarily used for their anti-inflammatory effects in disorders of many organ systems. Corticosteroids inhibit the inflammatory response to a variety of inciting agents and probably delay or slow healing. They inhibit the edema, fibrin deposition, capillary dilation, leukocyte migration, capillary proliferation, fibroblast proliferation, deposition of collagen, and scar formation associated with inflammation. Corticosteroids are thought to act by the induction of phospholipase A2 inhibitory proteins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of their common precursor arachidonic acid.

In the primary PD study in rabbits, there was a significant inhibition of VEGF-induced responses (VEGF-mediated blood-aqueous barrier and blood-retinal barrier breakdown, and vasculopathy) through six weeks with intravitreal injection of 1 mg triamcinolone acetonide gel suspensions, and through 22 weeks with intravitreal injection of 4 mg triamcinolone acetonide gel suspensions.

C. Nonclinical safety issues relevant to clinical use

There are no drug-related safety issues relevant to clinical use.

**APPEARS THIS WAY
ON ORIGINAL**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 22-220

Review number: 000

Sequence number/date/type of submission: 000/August 15, 2007/Commercial

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Allergan, Inc., 2525 Dupont Drive, P.O. Box 19534, Irvine, CA

Manufacturer for drug substance: _____

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Reviewer name: Zhou Chen

Division name: Division of Anti-Infective and Ophthalmic Products

Review completion date: March 26, 2008

Drug:

Trade name: Travaris™

Generic name: Triamcinolone acetonide

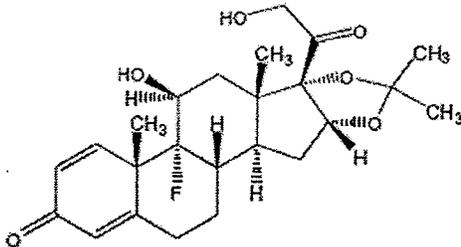
Code name: AGN-206230

Chemical name: 9-Fluoro-11β, 16α, 17, 21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16, 17 acetal with acetone

CAS registry number: 76-25-5

Molecular formula/molecular weight: C₂₄H₃₁FO₆, MW: 434.51

Structure:



Relevant INDs/NDAs/DMFs: IND _____ NDA 14-901, DMFs _____

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Drug class: Corticosteroid

Indication: Sympathetic ophthalmia, temporal arteritis, uveitis, and ocular inflammatory conditions unresponsive to topical corticosteroids

Clinical formulation

Component	Concentration (%)	Quantity per dose (mg)	Function
Triamcinolone acetonide	8.0	4.0	Drug substance
Sodium hyaluronate	2.3	1.2	
Sodium chloride	0.63	0.32	
Dibasic sodium phosphate	0.30	0.15	
Monobasic sodium phosphate	0.04	0.02	
Water for injection	qs to 100	qs to 50	

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Route of administration: Intravitreal injection

Proposed use: 50 µL (0.05 mL) per eye, delivering 4 mg triamcinolone acetonide to the vitreous humor

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

Studies reviewed within this submission:

Pharmacology:

Primary pharmacodynamics

Bio-05-487: Pharmacologic Evaluation of Intravitreal 1 mg (2%; #9633X) and 4 mg (8%; #9634X) Triamcinolone Gel Suspensions in Rabbits

PK:

Absorption:

PK-03-252: Comparison of Ocular and Systemic Pharmacokinetics of a 4% (4 mg) and 16% (16 mg) Prototype Triamcinolone Acetonide Formulation to that of 4% Kenalog[®] for 3 Months Following Intravitreal Injection in New Zealand Rabbit Eyes

PK-05-039: Toxicokinetic Analysis of Triamcinolone Acetonide for Allergan Study No. TX04040 Entitled "AGN 206230: Mammalian Erythrocyte Micronucleus Test"

PK-05-083: Toxicokinetic Analysis of Triamcinolone Acetonide for Allergan Study No. TX04022 Entitled "Triamcinolone: 6-Month Intravitreal Toxicity Study in Rabbits"

PK-07-024: 6-Month Retina Pharmacokinetics of Triamcinolone Acetonide Following Intravitreal Injection of 2% (1 mg) and 8% (4 mg) Triamcinolone Acetonide Injectable Gel Suspension in New Zealand White Rabbit Eyes

PK-05-047: Pharmacokinetics Analysis of Triamcinolone Acetonide (AGN 206230) in Rabbit Aqueous Humor, Vitreous Humor, and Plasma for Study PK-04-P031, Entitled "6-Month Ocular and Systemic Pharmacokinetics of Triamcinolone Acetonide Following Intravitreal Injection of 2% (1 mg) and 8% (4 mg) Triamcinolone Acetonide Injectable Gel Suspension in New Zealand White Rabbit Eyes"

Toxicology:

Single dose studies

TX03030: Triamcinolone Intravitreal Injection: 3-Month Ocular Toxicity Study in Rabbits

Repeated dose studies

TX04022: Triamcinolone Intravitreal Injection: 3-Month Ocular Toxicity Study in Rabbits

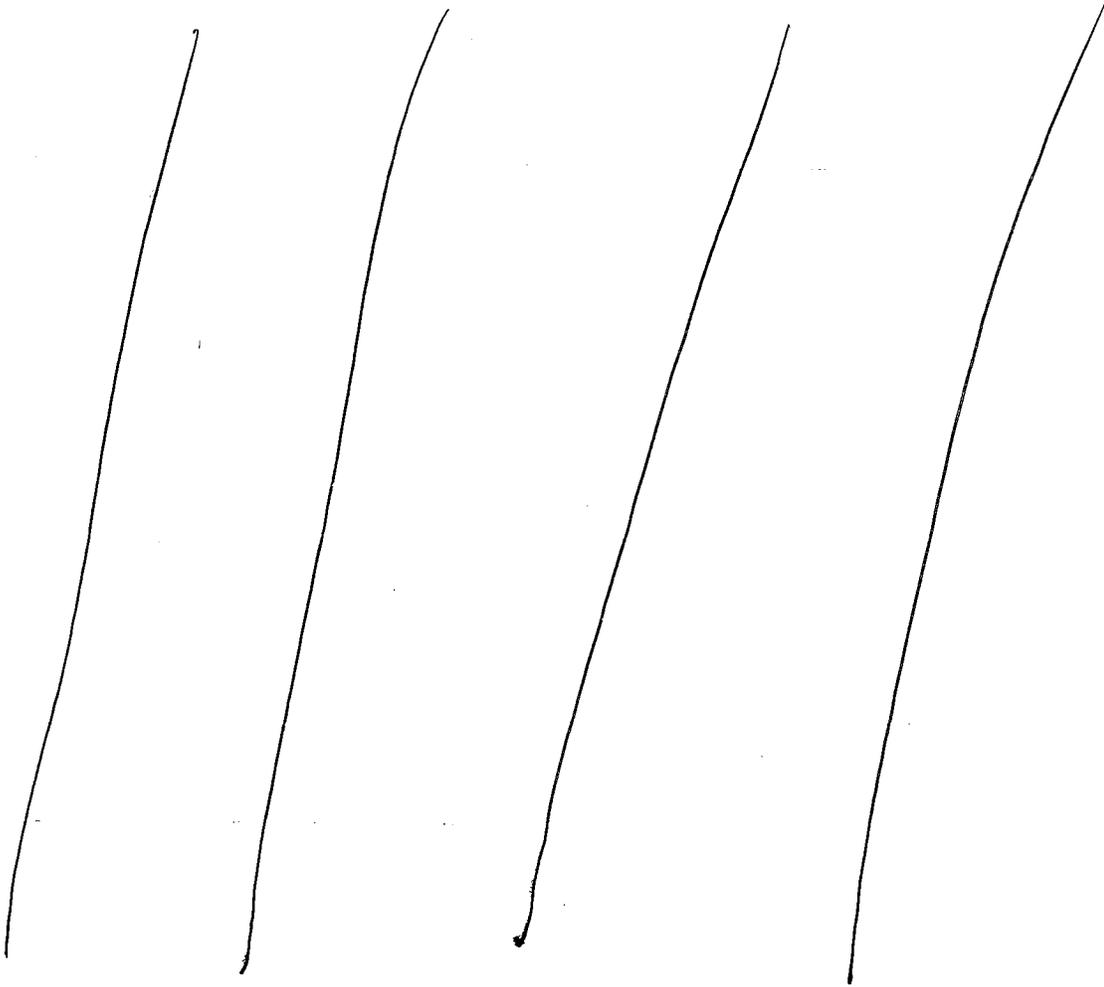
Genetic toxicology

TX04038: AGN 206230: Bacterial Reverse Mutation Assay with an Independent Repeat Assay

TX04039: AGN 206230: *In vitro* Mammalian Chromosome Aberration Test

TX04040: AGN 206230: Mammalian Erythrocyte Micronucleus Test

Studies not reviewed within this submission:



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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Only one primary PD study was submitted. In this study, there was a significant pharmacologic inhibition of VEGF-induced responses through six weeks with intravitreal injection of 1 mg triamcinolone acetonide gel suspensions, and through 22 weeks with intravitreal injection of 4 mg triamcinolone acetonide gel suspensions.

2.6.2.2 Primary pharmacodynamics

Bio-05-487: Pharmacologic Evaluation of Intravitreal 1 mg (2%; #9633X) and 4 mg (8%; #9634X) Triamcinolone Gel Suspensions in Rabbits

The purpose of this study was to evaluate the efficacy and duration of action of intravitreal 1 and 4 mg triamcinolone acetonide gel suspensions in a rabbit model of VEGF-mediated blood-aqueous barrier (BAB) and blood-retinal barrier (BRB) breakdown. Female Dutch Belt rabbits were randomly assigned into no treatment (control; 12 eyes), intravitreal injection of 1 mg triamcinolone acetonide gel suspensions (8 eyes), or intravitreal injection of 4 mg triamcinolone acetonide gel suspensions (10 eyes) groups. Since the VEGF responses are transient and return to baseline by one week, the drug's duration of action was determined by injecting VEGF intravitreally at six time points over a 7.5 month period (Weeks 2, 6, 10, 14, 22, and 30). Sodium fluorescein was administered intravenously (11.75 mg/kg) 48-hr after the VEGF injection, and BRB and BAB integrity were measured using scanning ocular fluorophotometry. BAB breakdown was measured by anterior chamber fluorophotometry, and BRB was measured by vitreal fluorophotometry and subjective grading of fluorescein angiograms. In addition, VEGF-induced changes in vessel caliber and tortuosity ($\Delta VC-T$) were assessed by subjective scoring of fundus images. The results are summarized below.

At the dose of 1 mg, triamcinolone acetonide gel suspensions had no effect on VEGF-induced BAB breakdown at any time point compared to control eyes.

At the dose of 1 mg, triamcinolone acetonide gel suspensions significantly inhibited VEGF-induced BRB and $\Delta VC-T$ through 6 weeks.

At the dose of 4 mg, triamcinolone acetonide gel suspensions significantly inhibited VEGF-induced BAB breakdown at 2, 6, and 14 weeks.

At the dose of 4 mg, triamcinolone acetonide gel suspensions significantly blocked VEGF-induced angiographic BRB breakdown through 14 weeks and fluorophotometric BRB breakdown and $\Delta VC-T$ through 22 weeks.

In conclusion, significant pharmacologic inhibition of VEGF-induced responses through six weeks with a single ITV injection of 1 mg triamcinolone acetonide, and through 22 weeks with a single ITV injection of 4 mg triamcinolone acetonide.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

In NZW rabbits following an ITV administration of triamcinolone acetonide (TA), high exposures to TA were seen in the vitreous humor and retina. The 4% TA prototype formulation generated similar intraocular TA concentrations compared to Kenalog®-40 at the same dose. The systemic exposure to TA following ITV injection was low (0.004% or less of the vitreous C_{max} values for all formulations).

2.6.4.2 Methods of Analysis

See descriptions under individual study reviews.

2.6.4.3 Absorption

PK-03-252: Comparison of Ocular and Systemic Pharmacokinetics of a 4% (4 mg) and 16% (16 mg) Prototype Triamcinolone Acetonide Formulation to that of 4% Kenalog® for 3 Months Following Intravitreal Injection in New Zealand Rabbit Eyes

Report N°: PK-03-252

Study N°: PK-03-P028

In-Life Test Facility: _____

Bioanalytical Facility: Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92623

GLP: No

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The purpose of this study was to determine the intraocular and systemic PK of triamcinolone acetonide (TA) in female New Zealand White rabbits for 45 days (vitreous humor and retina) and 90 days (aqueous humor) following an ITV injection of TA. Forty-two animals received a single bilateral ITV injection of 100 μ L Kenalog®-40 (4 mg), 4% (4 mg) TA prototype or 16% (16 mg) TA prototype. At 1, 3, 10, 17, 31, 45 and 90 days post injection, animals were sacrificed (2 rabbits per time point) and ocular tissues (aqueous humor, retina, vitreous humor) and plasma were analyzed for TA. Samples were quantified using LC-MS/MS methods.

Results:

Ocular PK data are summarized in the table below. Following a single ITV injection of either 4 or 16% prototype formulation or Kenalog-40, TA was detected in the aqueous humor, vitreous humor and retina at the earliest time point of Day 1. The vitreous humor TA concentrations for the 4% TA prototype formulation and Kenalog-40 were similar from Day 1 to 45, whereas, the 16% TA prototype formulation decreased ~3-fold by Day 45. The retinal C_{max} for 4% and 16% TA prototypes and Kenalog-40 were similar for all formulations.

Ocular PK data (mean± SD for C_{max} and mean± SD for AUC)

Treatment	C _{max} (µg/ml)	T _{max} (day)	T _{1/2} (day)	AUC (µg·day/ml)
Vitreous humor				
Kenalog-40 (4 mg)	394±278	45	Not calculable (nd)	14100±1650
4% TA prototype (4 mg)	385±110	10	nd	12500±1070
16% TA prototype	1610±758	3	75.8	33400±3870
Retina				
Kenalog-40 (4 mg)	2860±4100	1	nd	26200±10300
4% TA prototype (4 mg)	2530±4140	1	6.1	9230±3530
16% TA prototype	1750±1310	3	nd	13300±3740
Aqueous humor				
Kenalog-40 (4 mg)	0.137±0.053	1	nd	2.35±1.06
4% TA prototype (4 mg)	0.319±0.152	1	18.3	2.36±0.23
16% TA prototype	0.310±0.240	3	nd	3.20±0.60

Plasma PK data are summarized in the table below. The plasma C_{max} values were 0.004% or less of the vitreous C_{max} values for all formulations.

Plasma PK data

Treatment	C _{max} (ng/ml)	T _{max} (day)	T _{1/2} (day)	AUC (ng·day/ml)
Kenalog-40 (4 mg)	7.48	1	Not calculable	Not determined
4% TA prototype (4 mg)	15.8	1	10.9	Not determined
16% TA prototype	17.1	1	37.1	Not determined

In conclusion, the 4% TA prototype formulation generated similar intraocular TA concentrations compared to Kenalog®-40 at the same dose. The systemic exposure to TA following ITV injection was low.

PK-05-039: Toxicokinetic Analysis of Triamcinolone Acetonide for Allergan Study No. TX04040
Entitled "AGN 206230: Mammalian Erythrocyte Micronucleus Test"

Report N^o: PK-05-039

In-Life Test Facility: _____

Bioanalytical Facility: Allergan Inc., 2525 Dupont Drive, Irvine CA

GLP: Yes

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This is the TK part of study TX04040 entitled "AGN 206230: Mammalian Erythrocyte Micronucleus Test". The study was originally conducted with ICR mice (N = 3/sex/group) receiving a single intraperitoneal injection (IP) at 0 (vehicle), 500, 1000, or 2000 mg/kg/day. The study was later expanded in which one group of ICR mice (3/sex/group) received a single IP injection with another group of ICR mice (N = 3/sex/group) receiving two consecutive daily IP injections of AGN 206230 solution at 0 (vehicle), 500, 1000, or 2000 mg/kg/day. Plasma samples for TK evaluation were collected from study mice (3/sex/group) at 1 hour post-dose of the initial study. For the supplemental study, plasma samples were collected from study mice at 1 hour post-dose of the first group and at 1 hour post-dose after the second dose administration of the second group. Plasma concentrations of AGN 206230 from the above treatment groups were determined using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method with a lower limit of quantitation of 1 ng/mL.

Results:

PK parameters for this study are summarized in the following table. AGN 206230 was bioavailable following single and two consecutive daily IP injections of AGN 206230 at doses of 0, 500, 1000, or 2000 mg/kg/day in mice.

Plasma AGN 206230 concentrations at 1-hr post dosing (mean ± SD)

	Study day	Dose (mg/kg/day)	Concentration (ng/mL)	N
Initial	1	500	8670 ± 1260	3 M/3 F
Initial	1	1000	12800 ± 4300	3 M/3 F
Initial	1	2000	12000 ± 2200	3 M/3 F
Supplemental	1	500	16500 ± 3700	3 M/3 F
Supplemental	1	1000	16100 ± 8100	3 M/3 F
Supplemental	1	2000	13500 ± 2200	3 M/3 F
Supplemental	2	500	9510 ± 3610	3 M/3 F
Supplemental	2	1000	13600 ± 3000	3 M/3 F
Supplemental	2	2000	16700 ± 4200	3 M/3 F

PK-05-083: Toxicokinetic Analysis of Triamcinolone Acetonide for Allergan Study No. TX04022 Entitled "Triamcinolone: 6-Month Intravitreal Toxicity Study in Rabbits"Report N^o: PK-05-083

In-Life and Bioanalytical Facility: Allergan Inc., 2525 Dupont Drive, Irvine CA

GLP: Yes

This is the TK part of the study TX04022 entitled "*Triamcinolone: 6-Month Intravitreal Toxicity Study in Rabbits*". Female NZW rabbits (10/group) received two ITV injections (50 µL/injection) of 0, 1, or 4 mg TA in the left eye on Days 1 and 92. Blood samples were collected from 10 rabbits/group/timepoint pre-dose and Days 2, 4, 10, 21, 30, 60, 91, 93, 95, 101, 113, 121, 151, and 182 for TK evaluation. Plasma concentrations of TA were determined using a validated LC-MS/MS method with a quantitation range of 0.2-20 ng/mL.

Results:

Results are summarized in the table below. Peak TA plasma concentrations were observed 24 hr after intravitreal injection of TA in both treatment groups and during both treatment periods. Plasma C_{max} values were similar between both treatment groups for both treatment periods. The extent of systemic exposure (AUC_{0-t}) appears to be dose proportional. Duration of plasma drug concentrations was longer for 4 mg dose group compared to the 1 mg dose group.

Plasma TA PK data

Dose (mg/eye)	C _{max} (ng/mL)	T _{max} (hr)	AUC _{0-t} (ng-hr/mL)
Period No. 1 (Days 0-91) – after first injection			
0	NC (not calculable)	NC	NC
1	3.96 ± 1.57	24 ± 0 *	12.5 ± 5.4
4	3.28 ± 0.71	24 ± 0 *	51.1 ± 10.1
Period No. 2 (Days 91-182) – after second injection			
0	NC	NC	NC
1	3.04 ± 1.63	24 ± 0 *	10.8 ± 7.4
4	2.92 ± 0.53	24 ± 0 *	51.7 ± 16.4

*First sample timepoint following injection

PK-07-024: 6-Month Retina Pharmacokinetics of Triamcinolone Acetonide Following Intravitreal Injection of 2% (1 mg) and 8% (4 mg) Triamcinolone Acetonide Injectable Gel Suspension in New Zealand White Rabbit EyesReport N^o: PK-07-024

Study #: PK-04-P031

In-Life Facility: _____
 Bioanalytical Facility: Allergan, Inc
 GLP: Yes

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The purpose of this study was to compare the ocular and systemic pharmacokinetics of triamcinolone acetonide (TA) following a single unilateral ITV injection of 2% (1 mg) and 8% (4 mg) TA injectable gel suspensions in NZW rabbit eyes. Seventy-two female rabbits were intravitreally injected with one of two TA doses (2% or 8%) to monitor retinal pharmacokinetics. Rabbits (4/group/timepoint) were sacrificed on Days 2, 4, 11, 32, 64, 92, 121, 151, and 183 for retinal drug levels. Samples were quantified using an LC-MS/MS method. The pharmacokinetics of TA in aqueous humor, vitreous humor and plasma were reported separately (study report PK-05-047).

Results:

Results are summarized in the table below. Following a single ITV injection of either 2% or 8% TA gel suspension, TA was detected in the retina at the earliest timepoint of Day 2. Mean retinal maximal concentrations for 2% and 8% TA gel suspensions were 301 µg/g (Day 2) and 4720 µg/g (Day 2), respectively. The retinal drug levels for the 2% and 8% dose were detectable up to Day 32 (10.3 µg/g) and Day 64 (5.85 µg/g), respectively. There was contralateral diffusion noted especially at the higher dose (4 mg) level, possibly due to systemic absorption and distribution to the contralateral eye.

Retinal TA PK data (mean ± SD for C_{max} and mean ± SE for AUC)

Dose (mg/eye)	C _{max} (µg/mL)	T _{max} (days)	T _{1/2} (day)	AUC _{0-t} (µg-day/mL)	AUC interval (days)
2% TA gel suspension	301± 489	2	14.2	1220 ± 547	0-32
8% TA gel suspension	4720± 5270	2	9.81	14300 ± 5350	0-64

In conclusion, the 2% (1 mg) and 8% (4 mg) TA gel suspension delivered high level retinal TA concentrations up to 32 and 64 days, respectively.

PK-05-047: Pharmacokinetics Analysis of Triamcinolone Acetonide (AGN 206230) in Rabbit Aqueous Humor, Vitreous Humor, and Plasma for Study PK-04-P031, Entitled "6-Month Ocular and Systemic Pharmacokinetics of Triamcinolone Acetonide Following Intravitreal Injection of 2% (1 mg) and 8% (4 mg) Triamcinolone Acetonide Injectable Gel Suspension in New Zealand White Rabbit Eyes"

Report N^o: PK-05-047
 Study #: PK-04-P031
 In-Life Facility: _____
 Bioanalytical Facility: Allergan, Inc
 GLP: Yes

b(4)

The purpose of this study was to compare the ocular and systemic pharmacokinetics of triamcinolone acetonide (TA) following a single unilateral ITV injection of 2% (1 mg) and 8% (4 mg) TA injectable gel suspensions in NZW rabbit eyes. Seventy-two female rabbits were intravitreally injected with one of two TA doses (2% or 8%). Rabbits (4/group/timepoint) were sacrificed on Days 2, 4, 11, 32, 64, 92, 121, 151, and 183 for aqueous humor (AH), vitreous humor (VH) and plasma drug levels. Samples were quantified using validated LC-MS/MS methods with assay range for TA of 0.2-20 ng/mL in plasma, 1-500 ng/mL in AH and 0.4-100 µg/mL in VH.

Results:

Results are summarized in the table below. Following a single intravitreal injection of either 2 or 8% TA gel suspension, TA was detected in the AH, VH and plasma at the earliest time-point of Day 2. No contralateral diffusion of TA to the untreated eyes was detected in AH. The AH drug levels for the 2% and 8% doses were detectable up to Day 32 (4.15 ng/mL) and Day 151 (3.55 ng/mL), respectively. The area under the AH concentration time curve ($AUC_{0-t_{last}}$) was dose-dependent.

Following intravitreal injection of 2% and 8% TA gel suspensions, VH concentration of TA declined exponentially from 444 $\mu\text{g/g}$ (57.6% dose remaining) at 2 days postdose to 22.1 $\mu\text{g/g}$ (3.4% dose remaining) by 32 days post dose and from 1460 $\mu\text{g/g}$ (51.2% dose remaining) at 2 days to 33 $\mu\text{g/g}$ (1.3% dose remaining) by 151 days post dose, respectively. No contralateral diffusion of TA to the untreated eyes was detected in VH at all timepoints except for the 8% dose on Day 2 (0.306 $\mu\text{g/g}$).

Plasma TA levels were very low and were detected for the 2 and 8% doses up to Day 11 and Day 64, respectively.

TA PK data (mean \pm SD for C_{max} and mean \pm SE for AUC)

	2% TA gel suspension				8% TA gel suspension			
	C_{max} ($\mu\text{g/mL}$)	T_{max} (days)	$T_{1/2}$ (day)	AUC_{0-t} ($\mu\text{g}\cdot\text{day/mL}$)	C_{max} ($\mu\text{g/mL}$)	T_{max} (days)	$T_{1/2}$ (day)	AUC_{0-t} ($\mu\text{g}\cdot\text{day/mL}$)
Aqueous H.*	27.6 \pm 12.7	2	12.4	328 \pm 45	29.5 \pm 11.3	11	94.1	1310 \pm 178
Vitreous	567		8.57	3410 \pm 210	1590		32.8	68800 \pm 2940
Plasma*	4.12 \pm 0.25	2	3.11	18.1 \pm 0.9	3.59 \pm 0.87	2	16.2	83.6 \pm 12.4

* C_{max} and AUC units are ng/mL and ng-day/mL, respectively.

In conclusion, TA concentration was detected in ocular tissues of the animals treated with 2% (1 mg) and 8% (4 mg) TA gel suspensions up to 1 and 5 months postdose, respectively. The systemic exposure to TA following intravitreal injection was low and is expected to be relatively safe compared to systemic exposure of oral TA.

2.6.4.4 Distribution

No distribution studies were performed.

2.6.4.5 Metabolism

No metabolism studies were performed.

2.6.4.6 Excretion

No distribution studies were performed.

2.6.4.7 Pharmacokinetic drug interactions

No studies were provided.

2.6.4.8 Discussion and Conclusions

Following an ITV administration in NZW rabbits, high exposures to TA were seen in the vitreous humor and retina. The 4% TA prototype formulation generated similar intraocular TA concentrations compared to Kenalog[®]-40 at the same dose. The systemic exposure to TA following ITV injection was low.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General and ocular toxicology:

In the single ITV dose study in rabbits with TA in different formulations, no toxicologically significant ocular findings were observed with formulations containing 4% TA alone or in combination with 2% hyaluronic acid. Decreased ERG b wave, inflammatory responses (vitritis or chorioretinitis), and/or degenerative and necrotic lesions of the optic nerve head and retina were noted in other formulations (with

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Repeated intravitreal injection of triamcinolone acetonide (1 mg or 4 mg) in NZW rabbits at a 3-month interval was generally well tolerated. Mild to moderate ocular congestion was noted in both drug-treated and saline-treated groups but with a higher incidence in the treated groups.

Genetic toxicology:

AGN 206230 was negative in the Ames bacterial reversion test and chromosomal aberration assay in Chinese hamster ovary (CHO) cells. Positive results were noted in the *in vivo* micronucleus test with triamcinolone acetonide in mice.

2.6.6.2 Single-dose toxicity

TX03030: Triamcinolone Intravitreal Injection: 3-Month Ocular Toxicity Study in Rabbits

Study N^o: TX03030
 Compound: Kenalog-40 (4% TA), 2% hyaluronic acid (HA) + 4% TA, _____
 _____ + 4% TA, _____ + 4% TA, _____ + 4% TA, _____
 _____ 4% TA. _____ 4% TA, or _____
 _____ 4% TA
 Dose: 0.1 mL, 4 mg triamcinolone/injection
 Dosing regimen: Single dose
 Route: Intravitreal
 Animal: Female New Zealand White rabbit (6-month old, 3.58-4.31 kg, n = 3/group)
 Study site: Allergan, 2525 Dupont Drive, Irvine, CA 92612
 Study initiation: 6/19/2003

b(4)

GLP: No

The purpose of this non-GLP study was to evaluate the ocular effects of TA in various ophthalmic formulations when given by single intravitreal injection to female NZW rabbits followed by up to a 3-month observation period. Eight groups of female NZW rabbits (3/group) were given a single ITV injection of triamcinolone (4 mg) in various formulations in the left eye. The contralateral (right) eye received a similar volume of 0.9% sodium chloride. Rabbits were sacrificed and enucleated at week 7 (1 animal/group) or at 3-month (2 animals/group).

No drug-related mortality was observed. No drug-related effects on clinical observations, IOP, body weight or macroscopic observations were observed. Ocular irritation, including congestion, swelling and/or ocular discharge and tearing seen in both drug- and saline treated control eyes in all groups, was resolved within 2-3 weeks post injection. Small particles, possibly inflammatory cells, were observed intermittently in the vitreous with all drug formulations. Decreased ERG b-wave was seen at _____ -4% TA and neutrophil infiltration of the choroid was observed in eyes given _____ + 4% TA. Decreased ERG b-wave and vitritis were observed in eyes given 0.5% γ -CD + 4% TA, and chronic chorioretinitis was observed in eyes given _____ + 4% TA. Decreased ERG b-wave and incomplete pupillary response with _____ + 4%TA, and degenerative and necrotic lesions of the optic nerve head and retina were observed in eyes given \geq _____ + 4% TA. No toxicologically significant ocular findings were observed with formulations containing 4% TA alone or in combination with 2% HA.

b(4)

2.6.6.3 Repeated-dose toxicity

TX04022: Triamcinolone Intravitreal Injection: 3-Month Ocular Toxicity Study in Rabbits

Key study findings: Repeated intravitreal injections of triamcinolone acetonide (1 mg or 4 mg, 50 μ L/injection, left eye only) at a 3-month interval were generally well tolerated. Mild to moderate ocular congestion was noted in both drug-treated and saline-treated groups but with a higher incidence in the drug-treated groups.

Study no.: TX04022

Conducting laboratory and location: Allergan, 2525 Dupont Drive, Irvine, CA 92612

Date of study initiation: 8/20/2004

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Triamcinolone acetonide gel suspensions, 2% and 8%, formulation no. 9634X, lot no. 12330A1 for 8%; formulation no. 9633X, lot no. 12329A1 for 2%, purity \geq 99.9%

Control article: 0.9% sodium chloride injection

The purpose of this study was to evaluate the local toxicity following two intravitreal injections (at a 3-month interval) using the clinical formulations in female NZW rabbits. Each injection was followed by a 3-month observation period. A 3-month recovery period followed the second 3-month observation period.

Methods

Doses: 0.05 mL, 1 mg or 4 mg triamcinolone/injection, two injections at a 3-month interval

Species/strain: Female New Zealand White rabbit

Number/sex/group or time point (main study): 10 females/group

Route, formulation, volume, and infusion rate: Intravitreal injection (ITV, left eyes only), clinical formulations, 0.05 mL

Satellite groups used for toxicokinetics or recovery: Two animal/group underwent a 3-month recovery period.

Age: 4-month old

Weight: 2.9-3.2 kg

Group	N*	Dose (mg/left eye)	Volume injected (µL)	Right eye
1	10	0	50	Untreated
2	10	1	50	Untreated
3	10	4	50	Untreated

* After the 6-month treatment/observation period, two animals from each group underwent a 3-month recovery period.

Observation and Times:

Mortality: At least once daily

Clinical signs: At least once daily for 1 month following each intravitreal injection, and twice weekly thereafter throughout the observation and recovery periods

Body weights: Once weekly

Food consumption: Once weekly

Ocular observations: Each eye, once pretest, at least once daily for 1 week after each ITV injection, and once weekly thereafter throughout the observation and recovery periods.

Ophthalmic examinations: Both eyes were examined with a slit lamp biomicroscope and a direct or an indirect ophthalmoscope prior to treatment, monthly (up to 6 months) post first injection, and once at the end of the recovery period.

Intraocular pressure: Both eyes, prior to initiation of treatment (baseline), monthly (up to 3 months) post first and second injections, and once at the end of the recovery period

ERG: Both eyes, 2 times prior to the first injection (baseline), once 2 weeks post-first injection, once prior to the second injection (month 3), once 2 weeks and 3 months post-second injection, and one time at the completion of the recovery period.

Gross pathology: At the end of the treatment/observation period (6 months post-first injection, 8/group) or recovery period (9 months post-first injection, 2/group)

Organ weights: Not performed

Histopathology: Ocular tissues from all animals

TK: Blood samples were collected from all animals predose and on Days 2, 4, 10, 21, 30, 60, 91, 93, 95, 101, 113, 121, 151, and 182. Quantification of plasma drug concentrations was performed using LC-MS/MS methods.

Results:

Mortality: No drug-related mortality occurred in any group during the course of this study. On study Day 1, one Group 3 animal died under general anesthesia during the injection procedure. The death was not considered test article related, but was due to the anesthesia procedure. This animal was replaced.

Clinical observations: No drug-related effects on clinical observations were noted in any group during the study.

Gross ocular observations: Incidental, post-treatment findings related to the ITV injection procedure including mild to moderate ocular congestion were observed in both drug-treated and saline-treated groups, occurring mostly within 1 to 2 weeks post-injection. In general, the severity was comparable between the

drug-treated and saline-treated control groups; however, the frequency was slightly higher in two drug-treated groups compared to the control.

Conjunctival congestions seen in gross ocular observations

	Group 1				Group 2				Group 3			
	Treatment period		Recovery period		Treatment period		Recovery period		Treatment period		Recovery period	
Left eye	Total	% freq	Total	% freq	Total	% freq	Total	% freq	Total	% freq	Total	% freq
Congestion, +1	28	7	0	0	78	21	3	11	73	19	6	21
Congestion +2	17	4	0	0	9	2	0	0	3	1	0	0
Right eye	Total	% freq	Total	% freq	Total	% freq	Total	% freq	Total	% freq	Total	% freq
Congestion, +1	17	4	0	0	27	7	10	36	11	3	0	0
Congestion +2	0	0	0	0	1	>1	0	0	0	0	0	0

Ophthalmic examinations: No drug-related effects were noted during slit lamp or ophthalmoscopic examinations.

IOP and ERG: No toxicologically significant, drug-related changes were observed.

Body weights and food consumption: No toxicologically significant, treatment-related differences in body weights and food consumption were noted.

Necropsy: No toxicologically significant, drug-related macroscopic ocular and systemic changes were observed.

Histopathological examinations: No toxicologically significant ocular or periocular microscopic alterations were observed at 6 months or 9 months (recovery). Procedure-associated, non drug-related minimal to mild scar tissue formation characterized by focal scleral fibrosis was observed at ITV injection sites in all (8/8) saline (placebo) as well as 1mg and 4 mg TA-treated left eyes at 6 months. Minimal injection site scar tissue was still evident in 2/2 saline, 1/2 1 mg TA and 2/2 4 mg TA-treated eyes after a 3 month recovery period.

TK: Plasma TK data, summarized in the table below, showed that plasma C_{max} values were similar between both treatment groups for both treatment periods, and the extent of systemic exposure (AUC_{0-t}) appears to be dose proportional.

Plasma TK data (mean + SD)

Dose (mg/eye)	C_{max} (ng/mL)	T_{max} (days)	AUC_{0-t} (ng·day/mL)
Period No. 1 (Days 0-91) – after first injection			
0	NC (not calculable)	NC	NC
1	3.96 ±1.57	1 ±0	12.5 ±5.4
4	3.28 ±0.71	1 ±0	51.1 ±10.1
Period No. 2 (Days 91-182) – after second injection			
0	NC	NC	NC
1	3.04 ±1.63	1 ±0	10.8 ±7.4
4	2.92 ±0.53	1 ±0	51.7 ±16.4

In summary, female NZW rabbits were given two ITV injections (50 μ L/injection, 1 injection every 3 months) of 2% (1 mg) or 8% (4 mg) TA into the left eye of each rabbit and necropsied at 6 or 9 months. Ocular congestion was seen in all groups in both drug- and saline-treated eyes with a higher incidence in drug-treated eyes. In conclusion: repeated ITV injections of triamcinolone acetonide (1 mg or 4 mg, 50 μ L/injection, left eye only) at a 3-month interval were generally well tolerated with mild to moderate ocular

congestion noted in both drug-treated and saline-treated groups but with a higher incidence in the drug-treated groups.

6.6.6.4 Genetic toxicology

TX04038: AGN 206230: Bacterial Reverse Mutation Assay with an Independent Repeat Assay

Key findings: AGN 206230 was not considered mutagenic under the present testing conditions.

Study no.: TX04038

Conducting laboratory and location: _____

Date of study initiation: 10/12/2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: AGN 206230, Lot #: R14587, purity = 99.3%

b(4)

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA-1535, TA-1537, TA-98, TA-100 and TA-102

Doses used in definitive study: 25, 75, 250, 750 and 2500 µg/plate

Basis of dose selection: Precipitation and toxicity in the dose range finding assay

Negative controls: Ethanol

Positive controls: 2-Nitrofluorene (NF); 2-aminoanthracene (AA); Sterigmatocystin; Mitomycin; Sodium azide; 9-aminoacridine (9AA)

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)		Dose µg/plate (w/o S9)	
<i>Salmonella typhimurium</i>	TA-1535	AA	1.0	Sodium azide	1.0
	TA-1537	AA	1.0	9AA	75
	TA-98	AA	1.0	NF	1.0
	TA-100	AA	1.0	Sodium azide	1.0
	TA-102	Sterigmatocystin	10	Mitomycin	1.0

Incubation and sampling times: The tester strains were exposed to the test article via the plate incorporation or preincubation (for the independent repeat assay only). Following incubation at $37 \pm 2^\circ\text{C}$ for 48-72 hr, revertant colonies were counted. All doses of the test article, the vehicle controls and the positive controls were plated in triplicate. Revertant colonies were counted either by automated colony counter or by hand.

Criteria for positive results: For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3 times the mean vehicle control value. Data sets for tester strains TA98, TA100 and TA102 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2 times the mean vehicle control value.

Results

Preliminary toxicity study: The dose levels tested were 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 µg per plate. Precipitate was observed beginning at 3333 µg per plate. No appreciable toxicity was observed. Based on the findings of the preliminary toxicity assay, the maximum dose tested in the initial mutagenicity assay was 5000 µg per plate.

Study validity: In the initial mutagenicity assay via plate incorporation, the dose levels tested were 50, 150, 500, 1500 and 5000 µg per plate using a 50 µL plating aliquot. Precipitate was observed beginning at 1500 µg per plate. No appreciable toxicity was observed. The dose levels tested in the independent repeat assay were 25, 75, 250, 750 and 2500 µg per plate using a 25 µL plating aliquot. In the independent repeat assay, the maximum dose was 2500 µg per plate because a 25 µL aliquot is the maximum that can be used with a 60-minute preincubation. Precipitate was observed beginning at 750 µg per plate. No appreciable toxicity was observed. In both initial mutagenicity assay and confirmatory assay, positive control compounds caused positive responses, and the number of revertants per plate of the vehicle controls was within the historical range. The study was valid.

Study outcome: In both initial mutagenicity assay and independent repeat assay, AGN 206230 did not increase the number of revertants of *Salmonella typhimurium* (strains TA-1535, TA-1537, TA-98, TA-100 and TA-102). Therefore, AGN 206230 was not mutagenic under the present testing conditions.

TX04039: AGN 206230: In vitro Mammalian Chromosome Aberration Test

Key findings: AGN 206230 was negative for the induction of structural and numerical chromosome aberrations in CHO cells under the present testing conditions.

Study no.: TX04039

Conducting laboratory and location: _____

Date of study initiation: 10/11/2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: AGN 206230, Lot #: R14587, purity = 99.3%

b(4)

Methods

Strains/species/cell line: Chinese hamster ovary (CHO-K1) cells

Doses used in definitive study: 4-hr incubation with or without S9: 62.5, 125, 250, 500, 1000, 1500, and 2000 µg/mL; 20-hr incubation without S9: 62.5, 125, 250, 500, 1000, 1500, and 1700 µg/mL

Basis of dose selection: The results of the precipitation and cytotoxicity in a preliminary toxicity assay

Negative controls: Ethanol

Positive controls: Mitomycin (MMC), 0.1 and 0.2 µg/mL without S9 and cyclophosphamide (CP), 10 and 20 µg/mL with S9

Incubation and sampling times: In the non-activated study, the cells were exposed to the test article for 4 hr or continuously for 20 hr up to the cell harvest at $37\pm 1^\circ\text{C}$ in a humidified atmosphere of $5\pm 1\%$ CO_2 in air. In the S9 activated study, the cells were exposed for 4 hr. Cells were collected approximately 20 hr after the initiation of treatment.

No. of replicates: Duplicates were made for all cultures.

Counting method: A minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations.

Criteria for positive results: The test article was considered to induce a positive response when the percentage of cells with aberrations was increased in a dose-responsive manner with one or more concentrations being statistically significant ($p\leq 0.05$). However, values that were statistically significant but do not exceed the range of historic solvent controls may be judged as not biologically significant.

Results

Preliminary cytotoxicity test: CHO cells were exposed to solvent alone and to nine concentrations of test article ranging from 0.1736 to 1736 $\mu\text{g}/\text{mL}$ in the absence and presence of an S9 reaction mixture. Visible precipitate was observed at dose levels $\geq 173.6 \mu\text{g}/\text{mL}$. At the conclusion of the treatment period, visible precipitate was observed in treatment medium at dose levels $\geq 520.8 \mu\text{g}/\text{mL}$. Substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level with or without S9 in 4-hr exposure groups. Substantial toxicity was observed at 1736 $\mu\text{g}/\text{mL}$ in the non-activated 20-hr continuous exposure group. Based upon the results of the toxicity study, the dose levels selected for testing in the chromosome aberration assay were as follows:

4-hr incubation with or without S9: 62.5, 125, 250, 500, 1000, 1500, and 2000 $\mu\text{g}/\text{mL}$

20-hr incubation without S9: 62.5, 125, 250, 500, 1000, 1500, and 1700 $\mu\text{g}/\text{mL}$

Study validity: Acceptable controls, acceptable high dose and acceptable number of doses. The study was valid.

Study outcome: Results are summarized in the table below. In all groups, the percentage of cells with numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level.

In the non-activated 4 hr exposure group, the dose levels selected for microscopic analysis were 125, 250 and 500 $\mu\text{g}/\text{mL}$. The percentage of cells with structural aberrations was significantly increased above that of the solvent control at 500 $\mu\text{g}/\text{mL}$ ($p\leq 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p<0.05$). However, the percentage of cells with structural aberrations in the test article-treated group (4.5%) was within the historical solvent control range of 0.0% to 5.5%. Therefore, it is not considered to be biologically significant.

In the S9 activated 4 hr exposure group, the dose levels selected for microscopic analysis were 500, 1000 and 2000 $\mu\text{g}/\text{mL}$. The percentage of cells with structural aberrations was significantly increased above that of the solvent control at 1000 $\mu\text{g}/\text{mL}$ ($p\leq 0.05$, Fisher's exact test). The Cochran-Armitage test was negative

for a dose response ($p > 0.05$). However, the percentage of cells with structural aberrations in the test article-treated group (3.5%) was within the historical solvent control range of 0.0% to 5.0%. Therefore, it is not considered to be biologically significant.

In the non-activated 20 hr exposure group, the dose levels selected for microscopic analysis were 62.5, 125 and 250 $\mu\text{g/mL}$. The percentage of cells with structural aberrations was significantly increased above that of the solvent control at 250 $\mu\text{g/mL}$ ($p \leq 0.05$, Fisher's exact test). The Cochran-Armitage test was negative for a dose response ($p > 0.05$). However, the percentage of cells with structural aberrations in the test article-treated group (4.0%) was within the historical solvent control range of 0.0% to 5.5%. Therefore, it is not considered to be biologically significant.

Summary of the *in vitro* chromosomal aberration assay in CHO cells

Treatment	4-hr treatment without S9		4-hr treatment with S9		20-hr treatment without S9	
	Numerical	Structural	Numerical	Structural	Numerical	Structural
Ethanol	2.5	0	2.0	0	1.5	0.5
AGN 206230 ($\mu\text{g/mL}$)						
62.5					1.5	1.0
125	1.5	0			1.5	0.5
250	2.0	0.5			2.0	4.0*
500	3.0	4.5*	2.5	0.5		
1000			2.5	3.5*		
2000			3.0	2.0		
Historical control	0-7.5	0-5.5	0-11.0	0-5.0	0-7.5	0-5.5
Mitomycin C 0.1/0.2 $\mu\text{g/mL}$	2.0	17.0*			2.5	17.0*
Cyclophosphamide 10 $\mu\text{g/mL}$			0.5	15.0*		
Historical positive control	0-8.5	8.5-54.0	0-6.5	8.0-70.0	0-8.5	8.5-54.0

* Statistically significant

In conclusion, the positive and solvent controls fulfilled the requirements for a valid test. Under the conditions of the assay described in this report, AGN 206230 was negative for the induction of structural and numerical chromosome aberrations in CHO cells.

Reviewer's comments: Because of concerns with the positive results seen in drug-treated cells, the reviewer requested experts from CDER PTCC Genetic Toxicity Committee to perform an independent review. Dr. Mamata De from the CDER PTCC Genetic Toxicity Committee reviewed this study (see attached CDER PTCC Genetic Toxicity Committee review) and concluded that "under this experimental condition there is a slight trend for the test article related increase in the structural chromosomal aberration." The reviewing pharmacologist decided to keep the negative conclusion for this study since all results from drug-treated groups are within the historical control range.

TX04040: AGN 206230: Mammalian Erythrocyte Micronucleus Test

Key findings: At 2000mg/kg, AGN 206230 induced a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow.

Study no.: TX04040

Conducting laboratory and location: _____

Date of study initiation: 10/11/2004

GLP compliance: Yes

QA reports: yes (X) no ()

b(4)

Drug, lot #, and % purity: AGN 206230, Lot #: R14587 (purity = 99.3%) for the first three assays and R14829 (purity = 98.3%) for the confirmatory assay

Methods

Strains/species/cell line: ICR mice, 6-8 weeks old

Body weight (g)	Pilot toxicity study	Initial micronucleus study	Supplemental studies	Confirmatory study
Males	26.5-31.0	27.7-32.9	29.4-34.6	27.3-31.7
Females	20.0-22.4	21.5-24.5	20.4-26.1	19.7-23.2

Doses used in definitive study: See table below

Four studies were conducted: an initial study, two supplemental studies (first and second) and a confirmatory study. The study designs are summarized in the table below.

Study	Initial pilot study	Initial definitive study	Supplemental study 1	Supplemental study 2	Confirmatory study
Dose (mg/kg)	1, 10, 100, 1000, 2000	0, 500, 1000, 2000	0, 500, 1000, 2000	0, 500, 1000, 2000	0, 1000, 2000
Frequency, route	Single IP	Single IP	Single IP	Two IP	Single IP
Bone marrow collection after the last dose		24 (all doses) and 48 (2000 mg/kg) hr	24 (all doses) and 48 (2000 mg/kg) hr	24 hr	24 and 48 hr
Animals/group	2 males/group, and 5/sex for 2000 mg/kg	5/sex/group, 10/sex at 2000 mg/kg, 3TK mice/sex/group	5/sex/group, 10/sex at 2000 mg/kg, 3TK mice/sex/group	5/sex/group, 3TK mice/sex/group	5/sex/group for 24 hr and 10/sex/group for 48 hr sacrifices

Basis of dose selection: A pilot toxicity study

Negative controls: Corn oil

Positive controls: Cyclophosphamide (CP) 50 mg/kg, single dose IP injection

Incubation and sampling times: Animals were terminated at 24 or 48 hr after dosing and bone marrow samples were prepared. Positive control animals were sacrificed at 24 hr after dosing. The number of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) among 1000 erythrocytes (PCE + NCE) per animal was determined. The number of micronucleated polychromatic erythrocytes (MPCE) then was determined for 2000 PCE per animal.

Criteria for a positive response: A dose-responsive increase in micronucleated polychromatic erythrocytes was observed and one or more doses were statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables) at any sampling time. However, values that were statistically significant but did not exceed the range of historical negative or vehicle controls were judged as not biologically significant. The test article was judged negative if no statistically significant increase in micronucleated polychromatic erythrocytes above the concurrent vehicle control values and no evidence of dose response were observed at any sampling time.

Results

Pilot toxicity study: In the pilot study, no mortality occurred at any dose level. Lethargy was observed in male and female mice at 2000 mg/kg. All other animals appeared normal during the course of the study. Based upon these results, the highest dose for the micronucleus study was set at 2000 mg/kg.

TK: TK analysis of the plasma samples collected in the initial and two supplemental studies showed that AGN 206230 was detected in systemic circulation one hr following single or two consecutive daily IP injections at doses of 500, 1000 or 2000 mg/kg.

Study validity: The positive controls produced a significant increase in the number of MPCE. The number of MPCE from vehicle controls was within the historical control range. The study was valid.

Study outcome: In the initial definitive study, reductions in PCE/EC ratio of 28 and 31% were observed in males and females, respectively, at the 48-hr harvest at 2000 mg/kg. AGN 206230 induced a statistically significant increase in the incidence of MPCEs in males and females at 2000 mg/kg 48-hr post-dosing. The number of MPCEs ranged from 5 to 14 MPCEs/2000 PCEs in 1/5 males and 3/5 females. The sponsor decided to repeat the study based on the following considerations: Although this number of MPCEs was outside the historical vehicle control range (males: 3 MPCEs/2000 PCEs; females: 4 MPCEs/2000 PCEs), a potential positive response was seen only at 48 hrs post-dosing. Individual animal data in these groups showed that 6 of 10 mice did not exhibit a positive response. In addition, no dose-dependent increase or a statistically significant increase at 24-hr post-dosing was observed. The following supplemental micronucleus studies were performed

In the first supplemental study, up to 28% reductions in the ratio of PCE/EC were observed in the 24-hr test article groups and up to 45% in the 48-hr groups, suggesting bioavailability and myelotoxicity. A statistically significant increase in the number of MPCEs was observed in the female group at 500 mg/kg 24-hr post-dosing. However, this increase is attributed to one female mouse that had 9 MPCEs/2000 PCEs, while the other animals had 1 or 2 MPCEs/2000 PCEs. Statistically significant increases in the number of MPCEs were observed in males and females at 2000 mg/kg 48-hr postdosing. The sponsor did not consider the data indicated a clear positive response because of the following:

- a. Statistically significant result in female group was within the range of historical negative control data
- b. Only 1/5 females exhibited a potential positive response and
- c. Extreme individual variability in magnitude of the response in males (1 -12 MPCE/2000 PCE).

In the second supplemental study (two repeat IP injections with a 24-hr interval), reductions in the PCE/EC ratio were observed in male and female-test article groups (up to 43%) relative to the vehicle controls, suggesting the test article-related myelotoxicity. A statistically significant increase in the incidence of MPCEs was observed only in the female group at 2000 mg/kg 24 hr following the second administration. In this case, females had higher number of MPCEs (3, 5, 9, 16, and 17 MPCEs/2000 PCEs) relative to the concurrent vehicle control, but only 4 females had higher number in MPCEs relative to the historical negative control data.

Due to high individual animal variability in the number of MPCEs in the male and female groups at 2000 mg/kg in all studies, the decision was made to conduct a confirmatory assay using a more robust study design that included a 5 animals/sex/group at the 24-hr point and a 10 animals/sex/dose at the 48 hr time point. In the confirmatory study, reductions in the ratio of PCE/EC at 2000 mg/kg ranged from 15% to 44% indicating bioavailability and myelotoxicity to bone marrow. No significant increases in the number of

MPCEs were observed at 2000 mg/kg in males or females at 24-hours or males at 48-hours. A statistically significant increase in the number of MPCEs in female 2000 mg/kg group at 48 hr was observed. The sponsor believes that the increase was not considered biologically significant. Due to lack of biologically significant increase in the number of MPCEs at 2000 mg/kg, bone marrow from animals dosed with 1000 mg/kg was not evaluated.

In conclusion, IP administration of AGN 206230 at 2000 mg/kg induced a statistically significant increase in the incidence of MPCEs. The sponsor stated that these results were not reproduced in the confirmatory study, and concluded that based on the results and under the conditions of the confirmatory study, AGN 206230 at a dose of 2000 mg/kg did not induce a biologically significant or relevant increase in the incidence of MPCEs in the bone marrow of male and female ICR mice. However, the reviewer has concerns about the positive findings seen in different tests, and is surprised to see 4 tests were performed for one *in vivo* assay. The reviewer has requested a consultation from the CDER PTCC Genetic Toxicity Committee. The consultation, made by Dr. Mamata De, is attached with this review. The committee concludes that AGN 206230 at 2000 mg/kg produced a positive result in this *in vivo* micronucleus assay.

Summary of genotoxicity studies:

The sponsor claimed that AGN 206230 was negative in a battery of genotoxicity studies. The reviewer has concerns over the *in vitro* mammalian chromosomal aberration assay and *in vivo* micronucleus assay in mice due to the positive results, and has requested a consultation from CDER PTCC Genetic Toxicity Committee. The consultation, made by Dr. Mamata De, is attached with this review.

In Dr. Mamata De's consultation, it is concluded that there is a slight trend for the test article related increase in the structural chromosomal aberration, and the drug is positive in the *in vivo* micronucleus assay based on the statistically significant positive result at high doses.

The reviewing pharmacologist agrees with the Committee's conclusion that the drug is positive in the *in vivo* micronucleus assay. For the *in vitro* mammalian chromosomal aberration assay, the reviewer concludes that it should be negative since all findings with the drug were within the historical solvent control range.

2.6.6.5 Carcinogenicity

No carcinogenicity studies were submitted.

2.6.6.6 Reproductive and developmental toxicology

No reproductive studies were submitted.

2.6.6.7 Local tolerance

No local tolerance studies were submitted.

2.6.6.8 Special toxicology studies

No studies were submitted.

2.6.6.9 Discussion and Conclusions

This NDA submission is under 505(b)(2). The drug product submitted here is a new triamcinolone acetonide product with a preservative-free hydrogel suspension formulation presented in a pre-filled syringe. The application relies on published literature for safety and effectiveness and on the Agency's previous finding of safety and effectiveness for triamcinolone acetonide for Kenalog®-40.

Triamcinolone, a potent, relatively insoluble synthetic corticosteroid, has been marketed for many years as an anti-inflammatory and anti-allergic agent. The approved administration routes include oral administration (4 to 48 mg/day), intramuscular injection (40 to 80 mg), intra-articular injection (2.5 to 40 mg), topical application and inhalation (200 µg, 3 to 4 times daily). The highest dose for deep intramuscular injection can be 100 mg. The drug is well-tolerated systemically in humans. In this NDA application, no general toxicity and safety pharmacology studies were performed. The highest ocular dose will be 4 mg/eye, which is much lower than the approved clinical systemic dose. Clinical experience indicates TA is well-tolerated systemically in humans. It seems that systemic safety is not a concern.

To address local ocular toxicity, the sponsor performed two ocular toxicity studies in rabbits. The first one is a single dose study comparing TA with different formulations. Study results indicated that no toxicologically significant ocular findings were observed with formulations containing 4% TA alone or in combination with 2% hyaluronic acid. The second study was a repeat dose study in which TA was administered to the rabbit twice by ITV injections separated by a 3-month interval. The drug was generally well-tolerated. In both studies, reversible ocular irritation, including congestion, swelling and/or ocular discharge and tearing, was seen in both drug- and vehicle-treated animals, suggesting that the irritation might be related to the injection procedure. From the two ocular toxicity studies, it was concluded that there was no toxicologically significant, drug-related toxicity with triamcinolone acetonide by intravitreal injection.

Triamcinolone acetonide is an approved drug for many indications including severe acute and chronic allergic and inflammatory conditions. The drug has not been approved for intravitreal route; however, there are many clinical reports indicating that the route is safe and well tolerated in clinical practice. The use of intravitreal triamcinolone acetonide (Kenalog-40, 4-25 mg) to treat ocular inflammatory diseases including sympathetic ophthalmia, temporal arteritis, macular edema, and uveitis is now a therapeutic treatment performed by ophthalmologists around the world. The adverse events reported in clinical practice include intraocular pressure elevation, cataract formation, retinal detachment, vitreous hemorrhage and endophthalmitis. These complications may be related to the injection procedure or the corticosteroid suspension. Generally, the safety of the drug by the ITV route is supported by both nonclinical data and previous clinical experience.

The sponsor submitted study reports for a battery of genotoxicity studies. The drug was considered negative in two *in vitro* studies. For the *in vivo* micronucleus assay conducted in mice, both CDER PTCC Genetic Toxicity Committee and the reviewer consider the results were positive and should be stated in the labeling.

Considering the drug history and clinical experience, preclinical study results and the proposed indications, the reviewing pharmacologist believes that, from the nonclinical standpoint, the data are adequate for the approval of the drug, and no more nonclinical studies are needed.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Triamcinolone acetonide is an approved drug for many indications including severe acute and chronic allergic and inflammatory conditions. There is an over 40-year history of use of TA in the US with adequate demonstration of safety and efficacy for the treatment of sympathetic ophthalmia, temporal arteritis, uveitis, and ocular inflammatory unresponsive to topical corticosteroids. The safety of the drug by the ITV route is supported by both nonclinical data and previous clinical experience. From pharmacology/toxicology standpoint, an "approvable" was recommended with some labeling modifications.

Unresolved toxicology issues (if any): No

Recommendations:

This application is approvable from a nonclinical perspective with some modifications of labeling as revised in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section.

Suggested labeling:

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

No adequate studies have been conducted in animals to determine whether corticosteroids have a potential for carcinogenesis

Triamcinolone acetonide was not mutagenic or clastogenic in the Ames bacterial reversion test and chromosomal aberration assay in Chinese hamster ovary (CHO) cells. Positive results were noted in the in vivo micronucleus test with triamcinolone acetonide in mice.

b(4)

Steroids may increase or decrease motility and number of spermatozoa in some patients.

APPENDIX/ATTACHMENTS

CDER PTCC Genetic Toxicity Committee Consultation



FDA Center for Drug Evaluation and Research
White Oak, Building 22, 10903 New Hampshire Ave, Silver Spring, MD 20993

Date: February 6th, 2008

To: Zhou Chen, Ph.D.
Pharmacology/Toxicology Reviewer,
HFD110

Through: Timothy Robison, Ph.D
Chair, Genetic Toxicity Committee
CDER, FDA

From: Mamata De, Ph.D.
Pharmacologist, DAARP

Subject: Secondary review of the genotoxicity Studies entitled:
1. AGN 206230: Mammalian Erythrocyte Micronucleus Assay
(Study Report # AA 99MS.123.BTL); and
2. AGN 206230: In vitro Mammalian
Chromosomal Aberration Assay (Study
Report # AA 99MS.331.BTL)

Date Response Requested: 02/08/2008

Background:

AGN 206230 (fluorinated prednisolone derivative) is a synthetic corticosteroid. The applicant Allergen Inc. is developing the compound as an injectable formulation for the treatment of ocular inflammation (4 mg /50 µl gel suspension). In addition to its use in ophthalmics, the applicant also intends to develop AGN206230 as an intramuscular injection (40-80 mg for chronic duration) and an intra articular injection or the treatment of various inflammatory conditions which need corticosteroid therapy. The applicant conducted two clastogenicity studies as follows:

b(4)

1. AGN 206230: Mammalian Erythrocyte Micronucleus Assay (Study Report # AA 99MS.123.BTL);
2. AGN 206230: In vitro Mammalian Chromosomal Aberration Assay (Study Report # AA 99MS.331.BTL)

Following is the applicant's conclusion from the above mentioned studies as described in the toxicology written summary:

'2.6.6.4.3 In Vivo Mammalian System

TA was evaluated for clastogenic activity in mouse bone marrow following a single or two repeat IP injections in ICR mice at dose levels of 500, 1000 or 2000 mg/kg (Report TX04040). Dose levels of 500 and 1000 mg/kg did not induce a biologically relevant increase in micronucleated polychromatic erythrocytes (MPCEs) in the bone marrow. A dose level of 2000 mg/kg induced a statistically significant increase in the incidence of MPCEs in bone marrow; however, the results were not reproduced in the confirmatory study. Based on the results and under the conditions of the confirmatory study, AGN 206230 at 2000 mg/kg did not induce a biologically significant or relevant increase in the incidence of MPCEs in the bone marrow of male and female ICR mice.'

The primary reviewer Dr. Zhou Chen did not agree with the applicant's conclusion and requested the CDER Genetic Toxicology Subcommittee's feedback in the interpretation of applicant's data for the in vivo micronucleus assay.

Review of the Clastogenicity Assays by Genetic Toxicity Committee Member:

Dr Mamata De reviewed the *in vitro* and *in vivo* clastogenicity assays. Followings are the brief reviews of the above mentioned studies:

Evaluation of Study title 'AGN 206230: In vitro Mammalian Chromosomal Aberration Assay (Study Report # AA 99MS.331.BTL)':

In the chromosomal aberration assay, the Chinese hamster ovary cells were treated without metabolic activation for 4 hrs and 20 hrs and with metabolic activation for 4 hrs. The study is considered valid based on the data from the negative and positive control as indicated in the applicant's summary table below. The highest dose was appropriately selected based on the 50% reduction of the mitotic index. There was a statistically significant increase in the structural chromosomal aberration with or without metabolic activation at high dose.

In the chromosomal aberration assay, without metabolic activation for 4 hrs exposure, the mitotic index at the highest dose (500 µg/mL) was reduced (57 %) relative to the control. The dose levels selected for microscopic analysis were 125, 250 and 500 µg/mL. The incidences of structural aberration with 0, 125, 250, and 500 µg/mL were 0, 0, 0.5, and 4.5% respectively. The increase was statistically significant above that of the solvent control at 500 µg /mL ($p \leq 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$).

In the chromosomal aberration assay without metabolic activation for 20 hrs exposure, the mitotic index at the highest dose (250 µg/mL) was reduced (53 %) relative to the control. The dose levels selected for microscopic analysis were 62.5, 125, and 250 µg/mL. The incidences of structural aberration with 0, 62.5, 125, and 250 µg/mL were 0, 1, 0.5, and 4 % respectively. The increase was statistically significant above that of the solvent control at 250 µg /mL ($p \leq 0.05$, Fisher's exact test). The Cochran-Armitage test was negative for a dose response ($p > 0.05$).

In the chromosomal aberration assay with metabolic activation for 4 hrs exposure, the mitotic index at the highest dose (2000 µg/mL) was reduced (43 %) relative to the control. The dose levels selected for microscopic analyses were 0, 500, 1000, and 2000 µg/mL. The incidences of structural aberration with 0, 500, 1000, and 2000 µg/mL were 0, 0.5, 3.5, and 2 % respectively. The increase was statistically significant above that of the solvent control at 1000 µg /mL ($p \leq 0.05$, Fisher's exact test). The Cochran-Armitage test was negative for a dose response ($p > 0.05$).

Summary of Results: There was a statistically significant increase in the structural chromosomal aberration at high dose with metabolic activation for 4 hrs exposure and without metabolic activation for 4 and 20 hr exposure relative to that of the solvent control. The highest increase with metabolic activation for 4 hrs, without metabolic activation for 4 hrs, and without metabolic activation for 20 hrs was 3.5, 4.5, and 4.0% respectively. All these increases were within the historical control range for the solvent control (0-5.5%). Therefore, according to the applicant the increase is not biologically significant.

However, the increase in the structural chromosomal aberration without metabolic activation at 4 hr exposure was positive for dose response in the Cochran-Armitage test. There was also a test article related increase in the structural chromosomal aberration in lower doses compare to control without metabolic activation at 20 hrs exposure and with metabolic activation at 4 hrs. Thus in conclusion under this experimental condition there is a slight trend for the test article related increase in the structural chromosomal aberration.

**APPEARS THIS WAY
ON ORIGINAL**

TABLE 10
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Ethanol	-S9	4	10.9	200	200	0.000	±0.000	2.5	0.0
AGN 206230									
125	-S9	4	9.1	200	200	0.000	±0.000	1.5	0.0
250	-S9	4	7.9	200	200	0.005	±0.071	2.0	0.5
500	-S9	4	4.7	200	200	0.055	±0.269	3.0	4.5**
MMC, 0.2	-S9	4	3.3	200	100	0.280	±1.074	2.0	17.0**
Ethanol	+S9	4	12.9	200	200	0.000	±0.000	2.0	0.0
AGN 206230									
500	+S9	4	12.0	200	200	0.005	±0.071	2.5	0.5
1000	+S9	4	12.4	200	200	0.035	±0.184	2.5	3.5*
2000	+S9	4	7.3	200	200	0.020	±0.140	3.0	2.0
CP, 10	+S9	4	7.1	200	100	0.350	±1.445	0.5	15.0**
Ethanol	-S9	20	8.3	200	200	0.005	±0.071	1.5	0.5
AGN 206230									
62.5	-S9	20	7.0	200	200	0.010	±0.100	1.5	1.0
125	-S9	20	5.8	200	200	0.005	±0.071	1.5	0.5
250	-S9	20	3.9	200	200	0.045	±0.231	2.0	4.0*
MMC, 0.1	-S9	20	4.0	200	100	0.240	±0.622	2.5	17.0**

Evaluation of Study title 'AGN 206230: Mammalian Erythrocyte Micronucleus Assay'; Study Report # AA 99MS.123.BTL):

AGN206230 was tested in the mouse *in vivo* micronucleus to evaluate its clastogenic potential in four different experiments. It was noted that the drug lot (R14829) used in the 4th (last) test was different than the drug lot (R14587) with which the other three tests were conducted. The certificate of analysis for the drug lot# R14829 was not submitted with this study report and therefore can not be compared with the drug lot # 14587. The other experiments were considered valid based on the appropriate response exhibited by the positive and negative controls. The dose was appropriately selected based on the CNS related behavioral changes such as lethargy and piloerection observed at high dose and use of the limit dose of 2000 mg/kg. A reduction of polychromatic erythrocytes (PCE) compared to the total erythrocytes (TE) was noted indicating that the bone marrow was exposed to the test article. Also toxicokinetic analysis exhibited systemic absorption. The vehicle control used was corn oil; the test article formed a suspension in the corn oil. Extensive variation in the result were noted during the 24 hrs period may be due to the lack of uniformity of the test article suspension in the vehicle. Increased incidences of micronucleated polychromatic erythrocytes (MPCE) formations were evaluated/2000 PCE in the bone marrow to assess the clastogenicity. The results of the experiments are discussed below.

In the initial study no appreciable increase in the MPCEs were noted at 24 hrs with all of the doses that were tested (0, 500, 100, 2000 mg/kg). However, at 48 hrs a statistically significant increase in the MPCEs was

noted with the high dose (2000 mg/kg) in both males (\uparrow MPCEs: 13 w/2000 mg/kg vs 4 in control, $P \leq 0.05$) and females (\uparrow MPCEs: 39 w/2000 mg/kg vs 3 in control, $P \leq 0.05$) as depicted in the applicants table # 4 below. A reduction of PCE: TE 28% in male and 31% in females were observed at 2000 mg/kg in the male and female groups 48-hour post-dosing. These reductions demonstrate that the bone marrow was exposed to the test article and that the test article might have a myelotoxic effect on the bone marrow. In this experiment at 48 hr exposure with 2000 mg/kg all ten animals (5 males and 5 females) were observed to be positive for *in vivo* micronucleus formation compared to those of the 5 control animals (3 males and 2 females) suggesting an increased incidence of micronucleus formation. Also, the median number of the MPCEs were higher than the controls compare to those treated with the test article in both males (median: 1 in control vs 3 in test article) and females (median: 1 in control vs 7 in test article). This indicates a clear increase in the ratio micronucleus formation in the test article treated animals compared to those of the solvent controls at 48 hrs with high dose; since the applicant did not test the low doses at 48 hrs a NOAEL could not be determined in this experiment. No appreciable reduction in the ratio of PCE: TE was observed in the 24-hour test article-treated groups relative to the vehicle controls which might indicate that the bone marrow was not exposed to the test article during that time.

Table 4: Initial Micronucleus Study-Summary of Bone Marrow Micronucleus Analysis Following a Single Intraperitoneal Dose of AGN 206230 in ICR Mice

Treatment (20 mL/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean \pm SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
						Number per 1000 PCEs (Mean \pm SD)	Number per PCEs Scored
Corn oil	M	24	5	0.475 \pm 0.07	—	0.5 \pm 0.42	6 / 10000
	F	24	5	0.461 \pm 0.03	—	0.5 \pm 0.35	5 / 10000
AGN 206230 500 mg/kg	M	24	5	0.455 \pm 0.05	-4	0.5 \pm 0.35	5 / 10000
	F	24	5	0.462 \pm 0.02	0	0.4 \pm 0.22	4 / 10000
1000 mg/kg	M	24	5	0.473 \pm 0.02	0	0.3 \pm 0.45	3 / 10000
	F	24	5	0.463 \pm 0.02	0	0.8 \pm 0.27	8 / 10000
2000 mg/kg	M	24	5	0.451 \pm 0.03	-5	0.6 \pm 0.22	6 / 10000
	F	24	5	0.471 \pm 0.04	2	0.4 \pm 0.42	4 / 10000
Cyclophosphamide 50 mg/kg	M	24	5	0.349 \pm 0.02	-27	25.4 \pm 3.66	*254 / 10000
	F	24	5	0.361 \pm 0.02	-22	27.9 \pm 3.85	*279 / 10000
Corn oil	M	48	5	0.501 \pm 0.05	—	0.4 \pm 0.42	4 / 10000
	F	48	5	0.468 \pm 0.06	—	0.3 \pm 0.45	3 / 10000
AGN 206230 2000 mg/kg	M	48	5	0.363 \pm 0.02	-28	1.3 \pm 0.84	*13 / 10000
	F	48	5	0.323 \pm 0.06	-31	3.9 \pm 2.53	*39 / 10000

*Statistically significant, $p \leq 0.05$ (Kastobaum-Rowman Tables)

The sponsor decided to repeat the experiment to confirm the results of the initial study. In the second experiment, at 24 hrs, there was an increase in the micronucleus formation with the low (500 mg/kg) and mid dose (1000 mg/kg) but not at high dose (2000 mg/kg) compared to that of the control. There was extreme variability in the result, may be due to the fact that the test article is in suspension and not in complete solution. At 48 hrs, however, as observed in the initial study, an increase in the number of MPCEs was observed in both males (\uparrow MPCEs: 27 w/2000 mg/kg vs 5 in control, $P \leq 0.05$) and females (\uparrow MPCEs: 12 w/2000 mg/kg vs 4 in control, $P \leq 0.05$) as depicted in the applicants table # 8 below. A reduction of PCE: TE 35% in male and 45% in females was observed with 2000 mg/kg at 48-hour post-dosing. These reductions demonstrate that the bone marrow was exposed the test article and that the test article might be myelotoxic to the bone marrow. The positive result in the females in the second experiment with 2000 mg/kg at 48 hrs, although statistically significant compare to those of the experimental control, was within the historical control range (0-13/10,000 cells). The applicant concluded that the positive results were obtained from the males only and not from the females and therefore the results is not biologically significant. The increase in the frequency of MPCEs in five control females with 2000 mg/kg at 48 hrs was 1, 1, 0, 2, and 0. The increase in MPCEs in five test article related females at 48 hrs were 2, 2, 0, 3, and 5.

This data indicates a clear test article related increase in the micronucleus formation in the treated females at 48 hrs with high dose.

A reduction of PCE: TE was observed at all doses in both males and females at 24 hour in this study indicating exposure of the test article in the bone marrow. At 24 hrs, in females a test article related increase in the micronucleus formation was noted. The increase in MPCEs/10,000PCEs with 0, 500, 1000 mg/kg were 6, 15 (statistically significant, $P \leq 0.05$), and 10. The applicant believes that the significant change at the low dose was due to one outlier, the reviewer is in agreement with the applicant's conclusion. However, note that the number of MPCEs/10,000 in the five control animals were 2, 2, 0, 1, and 1 whereas in the females with 500 mg/kg dosing at 24 hrs were 1, 2, 1, 2, and 9 and that at the 1000 mg/kg were 2, 1, 0, 3, and 4. Note that all five females showed micronucleus formation with 500 mg/kg compared to 4/5 females at control indicating an increase in the incidence of micronucleus formation in addition. Also at mid dose a slight increase in the average number of micronucleus formation was noted compared to control (1.2 in control vs 2 in mid dose). All these evidence suggest a slight increasing trend in the micronucleus formation with low doses at 24 hrs in females. However, although statistically significant increase in females was observed at 48 hrs with 2000 mg/kg, no such changes were observed at 24 hrs. Similarly a slight increase in micronucleus formation compared to the control was noted in males at mid dose but not at low and high dose at 24 hrs.

The applicant did not test the low dose at 48 hrs; therefore like the initial study no NOAEL could be determined from this second study either for the males or the females.

Table 8: First Supplemental Study - Summary of Bone Marrow Micronucleus Analysis Following a Single Intraperitoneal Dose of AGN 206230 in ICR Mice

Treatment (10 ml/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean \pm SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
						Number per 1000 PCEs (Mean \pm SD)	Number per PCEs Scored
Corn oil	M	24	5	0.534 \pm 0.03	—	0.4 \pm 0.22	4 / 10000
	F	24	5	0.515 \pm 0.04	—	0.6 \pm 0.42	6 / 10000
AGN 206230 500 mg/kg	M	24	5	0.487 \pm 0.03	-9	0.2 \pm 0.27	2 / 10000
	F	24	5	0.441 \pm 0.07	-14	1.5 \pm 1.70	**15 / 10000
	M	24	5	0.419 \pm 0.06	-22	0.9 \pm 0.74	9 / 10000
	F	24	5	0.442 \pm 0.02	-14	1.0 \pm 0.79	10 / 10000
2000 mg/kg	M	24	5	0.382 \pm 0.06	-28	0.5 \pm 0.35	5 / 10000
	F	24	5	0.418 \pm 0.08	-19	0.3 \pm 0.45	3 / 10000
Cyclophosphamide*** 50 mg/kg	M	24	5	0.328 \pm 0.06	-39	21.0 \pm 5.88	*210 / 10000
	F	24	5	0.334 \pm 0.02	-35	20.9 \pm 7.07	*209 / 10000
Corn oil	M	48	5	0.518 \pm 0.06	—	0.5 \pm 0.35	5 / 10000
	F	48	5	0.537 \pm 0.04	—	0.4 \pm 0.42	4 / 10000
AGN 206230 2000 mg/kg	M	48	5	0.336 \pm 0.09	-35	2.7 \pm 2.02	*27 / 10000
	F	48	5	0.308 \pm 0.08	-45	1.2 \pm 0.91	**12 / 10000

*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

**Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables), but not biologically relevant

***CP administered only once and bone marrow was collected 24-hours post-dosing. CP data were shared between two studies

Because of the confusing data at 24 hrs, the applicant conducted a third study. In this 3rd experiment, the test article was administered twice, 24 hrs apart, the dosages tested were 500, 1000, 2000 mg/kg. The bone marrow for the evaluation of micronucleus was collected at 24 hrs after the second time administration of the test article. There was a slight increasing trend in the micronucleus formation with the low and mid dose and statistically significant increase in the micronucleus formation at high dose in females compared to that of the controls. The increase in the number of MPCEs was observed in both males (↑MPCE: 10 w/2000

mg/kg vs 7 in control) and females (\uparrow MPCEs: 50 w/2000 mg/kg vs 7 in control $P \leq 0.05$) as depicted in the applicants table # 11 below. Although the increases in males were not statistically significant the number of MPCEs/10,000 was consistently higher than those of the controls at all doses. A reduction of PCE: TE was observed in all doses after the two injections indicating the bone marrow exposure of the test article. The result indicates a test article related increase in the micronucleus formation under this experimental condition in males and females.

Table 11: Second Supplemental Study - Summary of Bone Marrow Micronucleus Analysis Following Two Intraperitoneal Dose Administrations of AGN 206230 in ICR Mice

Treatment 20 mL/kg/treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
						Number per 1000 PCEs (Mean +/- SD)	Number per PCEs Scored ¹
Corn oil	M	24	5	0.534 ± 0.05	—	0.7 ± 0.27	7 / 10000
	F	24	5	0.512 ± 0.05	—	0.7 ± 0.27	7 / 10000
AGN 206230 500 mg/kg/day	M	24	5	0.392 ± 0.06	-27	0.8 ± 0.27	8 / 10000
	F	24	5	0.427 ± 0.08	-17	0.8 ± 0.27	8 / 10000
1000 mg/kg/day	M	24	5	0.362 ± 0.06	-32	1.4 ± 0.42	14 / 10000
	F	24	5	0.323 ± 0.02	-37	1.1 ± 0.55	11 / 10000
2000 mg/kg/day	M	24	5	0.302 ± 0.03	-43	1.0 ± 1.00	10 / 10000
	F	24	5	0.370 ± 0.03	-28	5.0 ± 3.16	*50 / 10000
Cyclophosphamide** 50 mg/kg	M	24	5	0.328 ± 0.06	-39	21.0 ± 5.88	*210 / 10000
	F	24	5	0.334 ± 0.02	-35	20.9 ± 7.07	*209 / 10000

¹Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

**CP administered only once and bone marrow was collected 24-hours post-dosing. CP data were shared between two studies

Due to the variability in the dose response, the applicant decided to repeat the experiment with increased number of animals (10/sex/group in contrast to the three previous experiments where 5/sex/group was tested). In this 4th experiment, the animals were treated with the 0 and 2000 mg/kg. The bone marrows were tested for MPCEs after 24 hrs and 48 hrs with 2000 mg/kg. A statistically significant ($P \leq 0.05$) increase was noted in females with 2000 mg/kg at 48 hrs (7-fold increase in MPCEs compared to control). A reduction in PCE: TE was observed indicating the bone marrow exposure of the test article. Due to the absence of the data from low dose a NOAEL could not be established. The test article used in this experiment is different than what was used in the other 3 experiments and the certificate of analysis for the batch used in this experiment was not submitted in the study report. So it is not known whether the purity and the composition of the different batches of the test articles are comparable or not. Therefore it is unclear whether the differences in the results for the micronucleus formation in the males in this experiment compare to those of the other experiments are related to the purity and the composition of the test article or not.

Table 14: Confirmatory Study - Summary of Bone Marrow Micronucleus Analysis Following a Single Intraperitoneal Dose of AGN 206230 in ICR Mice

Treatment (20 mL/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean \pm SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
						Number per 1000 PCEs (Mean \pm SD)	Number per PCEs Scored ¹
Corn oil	M	24	5	0.516 \pm 0.07	—	0.2 \pm 0.45	2 / 10000
	F	24	5	0.598 \pm 0.05	—	0.1 \pm 0.22	1 / 10000
AGN 206230 2000 mg/kg	M	24	5	0.438 \pm 0.05	-15	0.2 \pm 0.27	2 / 10000
	F	24	5	0.436 \pm 0.04	-27	0.2 \pm 0.27	2 / 10000
Cyclophosphamide 50 mg/kg	M	24	5	0.371 \pm 0.05	-28	14.8 \pm 2.80	*148 / 10000
	F	24	5	0.401 \pm 0.03	-33	13.4 \pm 2.30	*134 / 10000
Corn oil	M	48	10	0.510 \pm 0.04	—	0.1 \pm 0.16	1 / 20000
	F	48	10	0.492 \pm 0.06	—	0.2 \pm 0.26	4 / 20000
AGN 206230 2000 mg/kg	M	48	10	0.333 \pm 0.05	-35	0.1 \pm 0.21	2 / 20000
	F	48	10	0.276 \pm 0.04	-44	0.7 \pm 0.35	**14 / 20000

* p \leq 0.05, Statistically significant (Kastenbaum-Bowman Tables)

** p \leq 0.05, Statistically significant (Kastenbaum-Bowman Tables), but not biologically relevant

Summary of Results: A significant increase (p \leq 0.05, Kastenbaum-Bowman's table) in the *in vivo* micronucleus formation with high dose of AGN 206230 (2000 mg/kg) at 48 hrs was noted in females (4/4 experiments). In males, a significant increase (p \leq 0.05, Kastenbaum-Bowman's table) in the *in vivo* micronucleus formation with high dose of AGN 206230 (2000 mg/kg) was noted at 48 hrs (2/4 experiments).

In the 3rd experiment, there was an increase at 24 hrs in the frequency of MPCEs in males after the two intraperitoneal administration of the test article, the increase, however, was not statistically significant. The increase in the number of MPCEs in males in this experiment is 10 which are within the historical control range. In the same experiment a slight test article related increase in the number of MPCEs were noted in both males and females with 1000 mg/kg indicating a test article related effect in the increase in the number of MPCEs.

In the 4th experiment, there was no increase in the number of MPCEs in males although the same experiment showed a 7-fold increase in MPCEs compared to those of the control.

The applicant believes that due to the difference in the males and females noted in the 2/4 experiment, the data is not biologically relevant.

However, it should be noted that except for the 4th experiment increases in the number of MPCEs were observed in all other experiment in both males and females. The reason for no changes in MPCEs in male is unclear and may even result from the difference in the test article batch (the sponsor should provide the certificate of analysis from all of the test article batches). No NOAEL could be established from the above experiments. The test article appeared to have a delayed effect in increasing the micronucleus formation may be due to the delay in the exposure of the test article in the bone marrow. Also, the delayed results might have occurred due to test article induced bone marrow suppression.

Table: Summarizing In Vivo Micronucleus Tests w/ AGN 206230

Treatment /Dose (mg/kg)	No. MPCEs/ 10,000 PCEs		Median MPCEs/2000 PCEs		Number of animals w/† No. MPCEs/PCEs		PCE:ECs/Change from Control (%)	
	Male	Female	Male	Female	Male	Female	Male	Female
Experiment 1 (pilot study): 24 hr incubation (single IP injection)								
0	6	5	1	1	4/5	4/5	-	-
500	5	4	1	1	4/5	4/5	-4	0
1000	3	8	1	2	2/5	5/5	0	0
2000	6	4	1	1	5/5	3/5	-5	2
Experiment 1 (pilot study): 48 hr incubation (single IP injection)								
0	4	3	1	1	3/5	2/5	-	-
2000	13*	39*	3	8	5/5	5/5	-28	-31
Experiment 2 (first supplement study): 24 hr incubation (single IP injection)								
0	4	6	1	1	4/5	4/5	-	-
500	2	15*	0	3	2/5	5/5	-9	-14
1000	9	10	2	2	4/5	4/5	-22	-14
2000	5	3	1	1	4/5	2/5	-28	-19
Experiment 2 (first supplement study): 48 hr incubation (single IP injection)								
0	5	4	1	1	4/5	3/5	-	-
2000	27*	12*	5	3	5/5	4/5	-35	-45
Experiment 3 (second supplement study): 24 hr incubation (two IP injection)								
0	7	7	1	1	5/5	5/5	-	-
500	8	8	2	2	5/5	5/5	-27	-17
1000	14	11	3	2	5/5	5/5	-32	-37
2000	10	50*	3	9	4/5	5/5	-43	-28
Experiment 4 (confirmatory study): 24 hr incubation (single IP injection)								
0	2	1	0	0	1/5	1/5	-	-
2000	2	2	1	1	2/5	2/5	-15	-27
Experiment 4 (confirmatory study): 48 hr incubation (single IP injection)								
0	1	4	0	0	1/10	4/10	-	-
2000	2	14*	0	2	2/10	9/10	-35	-44

Overall Conclusion and Discussion:

AGN 206230 is determined to be positive (statistically significant) in the *in vivo* micronucleus tests at 48 hrs with 2000 mg/kg in females. The test article is found to be positive in ¾ tests (2/4 of which were statistically significant) in males and therefore concluded to be weakly positive in males.

According to my knowledge, the positive findings with the *in vivo* micronucleus test are rare. The results from the *in vitro* chromosomal aberration assay showed statistically significant increase in the structural chromosomal aberrations compared to the experimental control. Therefore, the weight of evidence from the *in vitro* and *in vivo* clastogenicity test indicates that the test article do have a positive clastogenic effect in the non clinical studies in females. The test article's effect on males in a few tests is equivocal; however, with the weight of evidence the test article's effect on the micronucleus formation appeared to be weakly positive.

The clastogenic effects of the glucocorticoids are reported in the published literature as well as in the label of some of the marketed glucocorticoids.

In the published literature corticosteroids such as dexamethasone were observed to be positive in the *in vitro* and *in vivo* clastogenicity assay (Sing et al, 1994). This reviewer noted that marketed synthetic glucocorticoids were known to be positive in the genotoxicity test. Flucinolone acetonide (Derma Smoothie), another synthetic glucocorticoid was not tested in the genotoxicity test but generic comment as follows is reported in the labeling.

'Some corticosteroids have been found to be genotoxic in various genotoxicity tests (i.e. the *in vitro* human peripheral blood lymphocyte chromosome aberration assay with metabolic activation, the *in vivo* mouse bone marrow micronucleus assay, the Chinese hamster micronucleus test and the *in vitro* mouse lymphoma gene mutation assay).'

Betamethasone, another synthetic glucocorticoid used in veterinary medicine was also found to be positive in the micronucleus assay and the chromosomal aberration assay. Committee for Veterinary Medicine from EMEA evaluated Betamethasone's toxicity and the following excerpts show that the EMEA committee's assessment of the genotoxic results:

Negative results were obtained in an *in vitro* assay for gene mutation in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA102 and *Escherichia coli* WP2 *uvrA* and in an *in vitro* forward point mutation assay (HPRT locus) in of Chinese hamster ovary (CHO) cells. In an *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes, cultures treated in the presence of metabolic activation and sampled at 20 hours had significant increases in cells with structural and numerical aberrations; cultures sampled at 44 hours after treatment showed no increase in cells with structural or numerical aberrations and all tests carried out in the absence of metabolic activation gave negative results. An *in vivo* micronucleus test was carried out in which groups of 5 male and 5 female mice were given two intraperitoneal injections, 24 hours apart, of 0, 250, 500 or 1000 mg/kg bw/day and killed 24 or 48 hours after dosing. At the 48 hour sampling time, statistically significant increases in micronucleated polychromatic erythrocytes were observed in males (but not females) given 250 and 500 (but not 1000) mg/kg bw/day. Because the increases the numbers of micronucleated polychromatic erythrocytes were observed only in males and were still within the historical control range, it was considered this to be a negative result.

Note that the EMEA committee concluded that the betamethasone was negative in the genotoxicity testing. The conclusion was based on the fact that the high dose was observed to be negative for the micronucleus formation and the positive results were found only in males. It is interesting to note that the positive micronucleus findings in betamethasone were gender specific. As indicated above AGN206230 also appeared to have some gender specificity, however, unlike betamethasone, AGN206230 was more potent in its clastogenic effect in the females compare to males. The reason for the gender related effects of the synthetic glucocorticoids are not known. Studies to determine the enzymatic pathways for the metabolism of AGN206230 or its active metabolites have not been studies in human and or animals, therefore it is unknown whether the difference noted was due to the difference in the metabolic activation or not. Also note that the positive effect with betamethasone and AGN206230 was appeared to be delayed (48 hrs), suggesting either an indirect effect and/or delayed exposure of these synthetic glucocorticoids in the bone

marrow. Unlike betamethasone, AGN2063230, however, was appeared to be positive for micronucleus formation at high dose.

It was noted that endogenous glucocorticoids mediates the genotoxic effect of certain compounds such as morphine as indicated by the increase in micronucleus formation (Sawant et al 2001). This also suggests an indirect effect of glucocorticoids in clastogenicity.

All these above mentioned reports suggest that the clastogenicity findings with AGN 206230 are not an isolated observation, the applicant did not explore the mechanism of the clastogenicity findings. Jooston et al 2004, evaluated different mechanism for the clastogenic effect of the steroids, so far the reason for the clastogenic effect of glucocorticoids is not known. Glucocorticoids are immunosuppressive compound and may be indirectly involve in the tumor promotion and growth. An evaluation of clastogenicity related to immunosuppression and tumor promotion might be needed eventually to elucidate the indirect carcinogenic potential of the glucocorticoids.

The active ingredient of AGN 206230 is triamcinolone acetonide (TA). It is an intermediate acting glucocorticoid. It is currently marketed as an anti-inflammatory agent called 'Kenalog' (licensed by BMS Pharma) that can be administered in various dosage forms, including oral (4-48 mg/day), intramuscular (40-48 mg/day), intradermal (1 mg/injection site) or inhalation (200 µg, 3-4 times daily) and 'Nasacort' (licensed by Aventis Pharma). In the labeling for Kenalog, it is currently indicated that there are no adequate studies to determine whether the corticosteroids have a potential for carcinogenesis or mutagenesis. The label of Nasacort, reads as follows:

In a 2-year study in rats, triamcinolone acetonide caused no treatment-related carcinogenicity at oral doses up to 1.0 mcg/kg (approximately 1/50 of the maximum recommended daily intranasal dose in adults and children on a mcg/m² basis). In a 2-year study in mice, triamcinolone acetonide caused no treatment-related carcinogenicity at oral doses up to 3.0 mcg/kg (approximately 1/30 of the maximum recommended daily intranasal dose in adults and children on a mcg/m² basis).

No evidence of mutagenicity was detected from *in vitro* tests (a reverse mutation test in *Salmonella* bacteria and a forward mutation test in Chinese hamster ovary cells) conducted with triamcinolone acetonide.

It was noted that the label of Kenalog and Nasacort do not address clastogenicity. However, according to the carcinogenicity study results from Nasacort label, it is assumed that AGN206230 may not have any direct carcinogenic effect. *This NDA was submitted under 505(b) (2), therefore, the carcinogenicity results from the other product may not be reproduced in its label without the right of reference.* The label of Nasacort, however, indicate that TA is not be directly related to the carcinogenic effect.

At present the significance of the positive clastogenicity findings with AGN206230 is unknown. Therapeutic plasma concentration in human is often in the nanogram or pictogram per mL range whereas the positive finding in this study is in the mg/kg range in mice (HED=162 mg/kg). The applicant did not establish a NOAEL for the clastogenicity findings. Also, the data from the study report did not show whether the clastogenic effect was recovered or not.

In conclusion, TA is clastogenic in the non clinical studies but not positive in the carcinogenicity bioassay in rodents. The immunosuppressive property of the steroidal hormones in human and their tumorigenic activity are well known. It is however, not known how or if the clastogenicity is related indirectly to any of the tumorigenic activity noted in human with the steroidal hormones. In the absence of any mechanistic data it can not be concluded definitely whether the positive clastogenicity findings of AGN206230 is biologically relevant or not.

Recommendation:

AGN206230 is determined to be weakly positive in the *in vivo* micronucleus assay based on the statistically significant positive result at high doses. The positive clastogenicity findings of the compound may be reported in the label with the division's discretion.

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