

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-201s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO

NDA: 21-201

Submission date: July 10, 2009 (complete response to August 2, 2004 action letter)

Drug: polidocanol (Asclera)

Sponsor: Chemische Fabrik Kreussler & Co., GmbH

Indication: Treatment of varicose veins of the lower extremities

Reviewing Division: Division of Cardiovascular and Renal Products

Background Comments:

This NDA was originally submitted to the Division of Dermatology and Dental Products in 2003. The pharm/tox review (completed 5/6/2004) recommended that this NDA could be approved from the pharm/tox perspective. No additional nonclinical studies were recommended. The NDA was not approved for other reasons. The application is now in the Division of Cardiovascular and Renal Products. The pharm/tox reviewer and supervisor in this division also agree that the application may be approved from a pharm/tox perspective.

The original pharm/tox review from 5/6/2004 included suggestions on wording for labeling. These were not further discussed because the application was not approved.

Recommendations and Conclusions:


I concur with the Division pharm/tox conclusion that the nonclinical data support approval of this NDA and that no additional nonclinical studies are necessary at this time.

I recommend that the nonclinical sections of the labeling be edited based on the comments in the original pharm/tox review of this NDA. I have discussed this with the division pharm/tox supervisor and he agrees that genotoxicity and reproductive toxicity data should be included in the labeling and that the animal toxicology section can be deleted.

Below is recommended wording for the nonclinical sections of the labeling and the rationale for the changes:

2 Page(s) of Draft Labeling has been Withheld in Full immediately following this page as B4 (CCI/TS)



Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-21201	ORIG-1	CHEMISCHE FABRIK KREUSSLER AND CO GMBH	AETHOXYSKLEROL (POLIDOCANOL)0.5%/1% 

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/s/

PAUL C BROWN
12/24/2009



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-201
DATE RECEIVED BY CENTER:	0/0/04
DRUG NAME:	Asclera™ (polydocanol)
INDICATION:	treatment of varicose veins, lower extremities
SPONSOR:	Chemische Fabrik Kreussler & Co. GmbH
REVIEW DIVISION:	Division of CardioRenal Products (HFD-110)
PHARM/TOX REVIEWER:	William T. Link, Ph.D.
PHARM/TOX SUPERVISOR:	Albert DeFelice, Ph.D.
DIVISION DIRECTOR:	Norman Stockbridge, M.D., Ph.D.
PROJECT MANAGER:	Michael V. Monteleone

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


MEMO ON APPPROVABILITY

Application is made for the use of Asclera™ (polidocanol) for sclerotherapy of spider veins ([REDACTED], very small varicose veins, ≤ 1 mm in diameter) and reticular veins (small varicose veins [REDACTED] 1 to 3 mm) in the lower extremities.

In this reviewer's opinion, the application is approvable. The NDA was previously considered approvable, from a Pharm/Tox perspective, by the Division of Dermatologic and Dental Drug Products, in 2004 (review in DARRTS, authors: David Allen and Norman See). This reviewer is in agreement with their conclusions and recommendations. The labeling adequately addresses the need for technical training regarding the administration and use of Asclera, as well as the dangers inherent with intentional or accidental misuse such as delivery of large boluses into veins or arteries.

The following brief summary is excerpted from their review:

- A. Brief Overview of Nonclinical Findings: Polidocanol destroys endothelial cells local to injection site, resulting in replacement of the damaged vessel with connective tissue. The primary effects noted in toxicology studies involved damage at or near the injection site, including discoloration, necrosis, scarring, and ulceration. The effects may be attributed to the pharmacologic effect of polidocanol, which acts by damaging endothelium near the injection site when administered intravenously. Systemic effects following repeated intravenous dosing included minor hemolysis, which was probably also the result of the pharmacological actions of the drug substance, which acts to disrupt cell membranes. Substantial multiples of the clinical dose could not be administered in repeat-dose toxicology studies due to dose-limiting local reactions at the injection site. In a battery of genetic toxicology studies, polidocanol was negative in bacterial reverse mutation assays in *Salmonella* and *E. coli*, and in a micronucleus assay conducted in mice. Polidocanol induced numerical chromosomal aberrations in cultured newborn Chinese hamster lung fibroblasts in the absence of metabolic activation. These data suggest that polidocanol is a weak clastogen, but not a strong genetic toxicant. Polidocanol was not teratogenic in rats or rabbits, although reduced fetal survival was observed in rabbits in which the dams were dosed over gestation days 6-20. The embryocidal effect observed in rabbits was probably secondary to maternal toxicity. Polidocanol did not affect reproductive performance (fertility) or ability of rats to deliver and rear pups (perinatal development).
- B. Pharmacologic Activity: Polidocanol induces endothelial damage through nonspecific surfactant activity, resulting in platelet aggregation at the site of damage, causing a dense network of platelets, cellular debris, and fibrin. The network occludes the vein, and the vein is replaced by fibrous connective tissue.
- C. Nonclinical Safety Issues Relevant to Clinical Use: The product induces irritation and necrosis in the vicinity of the injection site. This is essential to the desired clinical effect, but could induce pain, inflammation, and scarring in sensitive individuals, particularly if extravasated. Polidocanol induced an embryocidal effect when administered to pregnant rabbits. This effect was probably secondary to maternal toxicity, but the labeling of the product should reflect these data.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-21201	ORIG-1	CHEMISCHE FABRIK KREUSSLER AND CO GMBH	AETHOXYSKLEROL (POLIDOCANOL)0.5%/1% 

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/s/

WILLIAM T LINK
11/18/2009

ALBERT F DEFELICE
11/18/2009

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-201

Review number: 1

Sequence number/date/type of submission: N-000 (RS)/29-SEP-2003
N-000 (BP)/17-NOV-2003
N-000 (BL)/07-APR-2004

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Chemische Fabrik Kreussler & Co., GmbH
Rheingastrasse 87-93
D-65203 Wiesbaden, Germany

Manufacturer for drug substance: Chemische Fabrik Kreussler & Co., GmbH
Rheingastrasse 87-93
D-65203 Wiesbaden, Germany

Reviewer name: David Allen, Ph.D.; Norman A. See, Ph.D.

Division name: Division of Dermatologic and Dental Drug Products

HFD #: 540

Review completion date: 28-APR-2004

Drug:

Trade name: Aethoxysklerol® 0.5%, 1.0%, (b) (4)

Generic names (list alphabetically): polidocanol

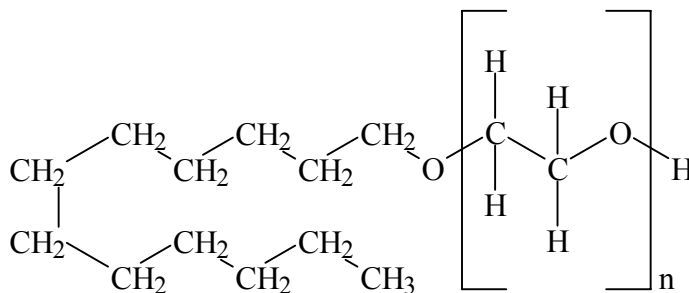
Code name: none

Chemical names: polyethylene glycol monododecyl ether; macrogol lauryl ether;
polyethylene glycol lauryl ether; lauromacrogol; polyoxyethylene lauryl alcohol ether

CAS registry number: 3055-99-0

Mole file numbers: not provided.

Molecular formula/molecular weights/structures: $C_{12}H_{25}(OCH_2-CH_2)_nOH$, $n = 1- \sim 22$



mean extent of polymerization (n) = 9
mean molecular weight = 600

Relevant INDs/NDAs/DMFs: In HFD-160

(b) (4)

(b) (4)

In HFD-520:

(b) (4)

In HFD-540: IND 35,139 (Kreussler) – active.

(b) (4)

Drug class: detergent sclerosing agent

Indication: Treatment of varicose veins of the lower extremities.

Clinical formulation (per 2 ml drug product):

Component	Aethoxysklerol 0.5%	Aethoxysklerol 1.0%	(b) (4)
polidocanol	10.00 mg	20.00 mg	(b) (4)
96% EtOH	84.00 mg	84.00 mg	(b) (4)
sodium phosphate	2.40 mg	4.80 mg	(b) (4)
c potassium phosphate	0.86 mg	1.70 mg	(b) (4)
water for injection	(b) (4)	(b) (4)	(b) (4)
	(b) (4)	(b) (4)	(b) (4)

Route of administration: intravenous

Proposed use: The product is proposed for use in the lower extremities for the sclerosis of varicose veins with a diameter of mm or less. In the pivotal clinical trials 2 ml of Aethoxysklerol constituted a single dose,

Therefore, as a realistic exposure for the purposes of comparison to the nonclinical studies,

Note, however, that the draft label of the product refers

(b) (4)

so I will be conservative in extrapolating between clinical and nonclinical exposures.

Introduction and drug history: Polidocanol was first introduced in 1936 as a local and topical anesthetic. One side effect of the drug when used for that purpose was sclerosis of small blood vessels. The drug was developed for use as a sclerosing agent, and Aethoxysklerol was registered for use in Germany as such in 1967. It reportedly has been used abroad for the treatment of telangiectasias of the lower limbs in human patients. Polidocanol is an amphiphilic, membrane-active compound that is classified as a surfactant. It is an aliphatic molecule that consists of a hydrophilic polyethylene oxide chain combined with a hydrophobic aliphatic fatty alcohol (dodecyl alcohol).

As detailed above, Aethoxysklerol contains up to mg polidocanol per 1 ml of an injectable solution. Based on studies in rats and rabbits, the Sponsor proposes that Aethoxysklerol treats varicose veins through a series of reactions. The drug substance, polidocanol, induces endothelial damage, which in turn causes platelet aggregation at the site of damage, resulting in a dense network of platelets, cellular debris, and fibrin. The network occludes the vessel which then obliterates the vein, and the vein is replaced by fibrous connective tissue. The sponsor states that the degree of this obliteration is directly proportional to the degree of endothelial damage. The sponsor also recognizes the possibility of recanalization of the treated vein, and thus periodic re-evaluation/re-treatment may be necessary. The Sponsor is proposing multiple concentrations of Aethoxysklerol so that the size of the vein will dictate the appropriate treatment. Aethoxysklerol 0.5% is indicated for varicose veins ≤ 1 mm in diameter; Aethoxysklerol 1.0% is indicated for varicose veins 1 to 3 mm in diameter; (b) (4)

The original NDA filing for Aethoxysklerol was under NDA 21-201 in 1999, but was withdrawn soon after due to what was described by the sponsor as, “issues with human pharmacokinetic data.” A

pre-NDA meeting was held on October 21, 2002 with the sponsor to discuss their re-filing of their NDA for Aethoxysklerol. In their preparation for NDA submission, the Sponsor encountered difficulties with the original manufacturing facility for polidocanol and Aethoxysklerol such that another commercial manufacturer had to be identified. For this reason, the polidocanol used in the nonclinical studies was manufactured at more than one facility. Only one single-dose toxicology study was performed with the to-be-marketed polidocanol. However, a multiple-dose toxicology bridging study was also performed to compare the toxicities associated with the polidocanol from each manufacturer.

Studies reviewed within this submission:

Single and repeat-dose toxicology

1. Investigation of Aethoxysklerol[®] on acute toxicity in rats after intravenous injection, study No. 024 TOX 94.
2. Investigation of Aethoxysklerol[®] on acute toxicity in rats after subcutaneous injection, study No. 023 TOX 94.
3. Investigation of Aethoxysklerol[®] on acute toxicity in rabbits after intravenous injection, study No. 026 TOX 94.
4. Investigation of Aethoxysklerol[®] on acute toxicity in rabbits after subcutaneous injection, study No. 025 TOX 94.
5. Single-dose subcutaneous and intravenous toxicity test of polidocanol in rats, study No. (b) (4)-89-RZAC-118.
6. Single-dose subcutaneous and intravenous toxicity test of polidocanol in mice, study No. (b) (4)-89-MZAC-119.
7. Acute toxicity of polidocanol by intravenous administration to Sprague-Dawley rats, study No. 11584/98.
8. Repeated dose study (4 weeks) in beagle dogs after i.v. injection of polidocanol, study No. (b) (4)-66.357-1.
9. Subacute toxicity test of ASK – 010 intravenous doses intermittently for 4 weeks and recovery test by discontinuation of doses for 4 weeks in dogs, study No. (b) (4)-87-DVSA-035.
10. Thirteen week intravenous toxicity study of ASK in rats, study No. 93208.
11. Thirteen week intravenous toxicity study of ASK in dogs, study No. 93209.
12. 7-day subchronic toxicity study of polidocanol by intravenous administration to Sprague-Dawley rats (Comparison of polidocanol from three different manufacturers), study No. 11128/1/98.

Genetic toxicology

1. Micronucleus test of Polidocanol Kreussler in bone marrow cells of the NMRI mouse by intravenous administration, study No. 16223/02.
2. Mutagenicity test of polidocanol, study No. ML-326A.
 - a. Reverse mutation assay.
 - b. Chromosomal aberration assay.
3. Mutagenicity testing of polidocanol in Ames *Salmonella*/microsome plate test, study No. 63318.

Reproductive and Developmental toxicology

1. Examination of the influence of polidocanol on the pregnant rat and the fetus by intravenous administration, study No. 8927/94.
2. Examination of the influence of polidocanol on the pregnant rabbit and the fetus by intravenous administration, study No. 8926/94.

3. Reproductive and developmental toxicity studies of polidocanol. (Fertility and early embryogenesis study in rats), study No. not clearly stated.
4. Reproductive and developmental toxicity studies of polidocanol. (Teratology study in rats), study No. not clearly stated.

Special Toxicology Studies

1. Intramuscular irritation test of polidocanol in rabbits, study No. ML-326B.

Studies not reviewed within this submission: The submission contained a number of photocopies of journal articles that were not specifically summarized in this review because they were judged to add no useful information to the database that was captured in the review.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

- A. Recommendation on Approvability: This NDA is approvable with respect to pharmacologic and toxicologic concerns.
- B. Recommendation for Nonclinical Studies: No additional nonclinical studies are recommended at this time.
- C. Recommendations on Labeling: The following changes in the draft labeling are recommended:

- 1. Clinical Pharmacology: The following paragraph should be deleted, as it does not pertain to clinical pharmacology:

[REDACTED]

- 2. Carcinogenesis, Mutagenesis, Impairment of Fertility: The text in this section should be stricken and replaced with:

"Long-term studies to evaluate carcinogenic potential have not been conducted with polidocanol. Polidocanol was negative in bacterial reverse mutation assays in *Salmonella* and *E. coli*, and in a micronucleus assay conducted in mice. Polidocanol induced numerical chromosomal aberrations in cultured newborn Chinese hamster lung fibroblasts in the absence of metabolic activation, [REDACTED]

Polidocanol did not affect reproductive performance (fertility) [REDACTED] of rats [REDACTED] when administered intermittently at dosages up to 10 mg/kg (approximately [REDACTED] maximum dose [REDACTED] on the basis of body surface area).

- 3. Pregnancy. The text in this section should be stricken and replaced with:

[REDACTED] Pregnancy category C. Polidocanol has been shown to have an embryocidal effect in rabbits when given in doses approximately equal to the human dose ([REDACTED] on the basis of body surface area). This effect may have been secondary to maternal toxicity. There are no adequate and well-controlled studies in pregnant women. [REDACTED]

- 4. [REDACTED]

II. Summary of Nonclinical Findings

- A. Brief Overview of Nonclinical Findings: Polidocanol destroys endothelial cells local to injection site, resulting in replacement of the damaged vessel with connective tissue. The primary effects noted in toxicology studies involved damage at or near the injection site, including discoloration, necrosis, scarring, and ulceration. The effects may be attributed to the pharmacologic effect of polidocanol, which acts by damaging endothelium near the injection site when administered intravenously. Systemic effects following repeated intravenous dosing included minor hemolysis, which was probably also the result of the pharmacological actions of the drug substance, which acts to disrupt cell membranes. Substantial multiples of the clinical dose could not be administered in repeat-dose toxicology studies due to dose-limiting local reactions at the injection site. In a battery of genetic toxicology studies, polidocanol was negative in bacterial reverse mutation assays in *Salmonella* and *E. coli*, and in a micronucleus assay conducted in mice. Polidocanol induced numerical chromosomal aberrations in cultured newborn Chinese hamster lung fibroblasts in the absence of metabolic activation. These data suggest that polidocanol is a weak clastogen, but not a strong genetic toxicant. Polidocanol was not teratogenic in rats or rabbits, although reduced fetal survival was observed in rabbits in which the dams were dosed over gestation days 6-20. The embryocidal effect observed in rabbits was probably secondary to maternal toxicity. Polidocanol did not affect reproductive performance (fertility) or ability of rats to deliver and rear pups (perinatal development).
- B. Pharmacologic Activity: Polidocanol induces endothelial damage through nonspecific surfactant activity, resulting in platelet aggregation at the site of damage, causing a dense network of platelets, cellular debris, and fibrin. The network occludes the vein, and the vein is replaced by fibrous connective tissue.
- C. Nonclinical Safety Issues Relevant to Clinical Use: The product induces irritation and necrosis in the vicinity of the injection site. This is essential to the desired clinical effect, but could induce pain, inflammation, and scarring in sensitive individuals, particularly if extravasated. Polidocanol induced an embryocidal effect when administered to pregnant rabbits. This effect was probably secondary to maternal toxicity, but the labeling of the product should reflect these data.

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: Destroys endothelial cells local to injection site, resulting in replacement of damaged vessel with connective tissue.

Mechanism of action: Disrupts membranes of endothelial cells by acting as a surfactant, resulting in cell death.

Drug activity related to proposed indication: Polidocanol induces endothelial damage through nonspecific surfactant activity, resulting in platelet aggregation at the site of damage, causing a dense network of platelets, cellular debris, and fibrin. The network occludes the vein, and the vein is replaced by fibrous connective tissue.

Secondary pharmacodynamics: NA

Pharmacology summary: Damages local endothelial cells through surfactant action, resulting in replacement of the damaged vessel with connective tissue.

Pharmacology conclusions: Induces destruction and replacement of portions of vessels near the injection site.

II. SAFETY PHARMACOLOGY:

Neurological effects: NA

Cardiovascular effects: NA

Pulmonary effects: NA

Renal effects: NA

Gastrointestinal effects: NA

Abuse liability: NA

Other: NA

Safety pharmacology summary: The "safety pharmacology" of the drug substance has not been investigated in a specific set of nonclinical studies dedicated to that purpose. However, many "safety pharmacology" issues were addressed in the toxicology studies discussed below.

Safety pharmacology conclusions: Clinical use of the drug product would not involve chronic exposure, and therefore systemic exposure to the drug substance would be minimized. In my opinion, safety pharmacology data, beyond those obtained in standard toxicology studies, are not essential to assessment of the safety of this product.

III. PHARMACOKINETICS/TOXICOKINETICS:

1. Study title: Pharmacokinetic study of polidocanol: tissue distribution, metabolism and excretion in rats

Study number: AE-1931

Performing organization: Tokai Research Laboratories of Daiichi Pure Chemicals Co, Ltd., Ibaraki, Japan

Drug lot and batch: ^{14}C -polidocanol (specific activity 2.34 MBq/mg), lot # CP-1660; unlabeled polidocanol, lot # 04463

Date of study: 3/22/94-2/24/95

GLP compliance: no

Study design:

Formulation: ^{14}C -polidocanol and unlabeled polidocanol dissolved in phosphate buffer containing 5% ethanol at concentrations of 0.2, 1 and 5 mg/ml.

Test animals: SD rats (SPF) 7-8 weeks of age, nursing rats were 10 days postpartum (mated at 9 weeks of age), 3/group except for whole body autoradiography in which 1 animal was sacrificed per time point.

a) single dose study in male rats - iv doses via tail vein at 0.4, 2, and 10 mg/kg

- female rats, iv via tail vein at 2 mg/kg

b) excretion of radioactivity in feces, urine, and expired air - male rats - single iv dose of 2 mg/kg

-female rats - single iv dose of 2 mg/kg

c) biliary excretion study in bile duct cannulated male rats, single iv dose of 2 mg/kg

d) quantitative tissue radioactivity levels in male rats, single iv dose of 2 mg/kg

e) multiple dose studies - male rats given up to 14 consecutive daily doses of 2 mg/kg iv.

- whole blood and plasma measurements

- excretion of radioactivity

- whole body autoradiography

f) quantitative tissue radioactivity levels after multiple dosing

g) radioactivity in breast milk

h) studies of metabolite profiles

Radioactivity was measured by liquid scintillation counting. Metabolite profiles were determined by HPLC.

Findings:

a) After a single iv dose of 2 mg/kg in male rats, the plasma concentration at 5 minutes (first sample) was 2.58 μg -equivalents/ml (whole blood concentration was 2.10 μg -equivalents/ml).

Polidocanol exhibited a biphasic time course with a terminal half-life of 15 hours (27 hours for

whole blood). Plasma concentrations were below the limit of detection at 96 hrs. The $AUC_{0-\infty}$ was 8.45 $\mu\text{g-equivalents}\cdot\text{hr}/\text{ml}$ (7.17 for whole blood). The time course was similar for doses of 0.4 or 10 mg/kg. The AUC appeared to have a linear relationship to dose. Similar results were seen in females.

b) After a single iv dose of 2 mg/kg in male rats, 63.8% of radioactivity was recovered in urine, 32.9% was recovered in feces, and 1.4% was recovered in expired air over 168 hours after dosing. 0.9% of radioactivity was retained in the body. Similar results were found for females.

c) In the biliary excretion study, by 48 hours, 25.3% of the radioactive dose was accounted for in the bile, 66.2% was recovered in urine, 3.1% recovered in feces, 0.4% was still associated with gastrointestinal contents, and 2.2% retained in body tissues. The presence of radioactivity in feces and gastrointestinal contents was presented as evidence for gastrointestinal secretion of the drug.

d) Five minutes after a single iv dose of 2 mg/kg in male rats, the plasma level was 2.78 µg-equivalents/ml and tissue radioactivity levels were highest in adrenal, liver, and kidney (2.2-2.9 times the plasma level), followed by pancreas, heart, and mandibular gland (*Reviewer's comment: It is unclear whether or not this refers to the mandibular salivary gland or if this is a mistranslation*) (1.5-1.7 times the plasma level). Levels in stomach, thyroid, pituitary, lung, Harderian gland, bone marrow, skeletal muscle, spleen, and whole blood were similar to plasma. Lower levels were found in eyeball, testis, and white fat (10-14% of plasma level). Levels in remaining tissues were 23-60% of plasma level.

At 2 hours, liver and kidney contained 5.1 and 2.3 times the concurrent plasma level of radioactivity (0.73 µg-equivalents/ml), respectively. By 6 hours, the level in most tissues was down to 2-14% of maximal levels. The exceptions were large intestine (83% of maximal levels), white fat (30%), and testis (22%). By 168 hours, all organs and tissues contained ≤7% of maximal levels, and levels in plasma and whole blood were below the level of detection.

In females, results were similar, except at 6 hours, when the large intestine level was 36% of maximal levels, and 16-18% of maximal levels were present in liver, ovaries, and white fat. By 168 hours, ovaries and white fat still contained 14% and 10% of maximal levels, respectively.

e) After 14 days of consecutive dosing, plasma radioactivity 24 hours after dosing reached a plateau at 5 days. Whole blood levels did not reach a plateau. After the last dose, the plasma level at 5 minutes after injection was 2.35 µg-equivalents/ml, and the terminal half life was 43 hours. The plasma AUC was 3.4 times that after single dose (5.9 times that for whole blood), or 28.8 µg-equivalents-hr/ml. There was an increase in the percent excreted in the feces and a decrease in the percent excreted in urine over time that reached a plateau after 7 days. By 168 hours after the last dose, 55.4% of the total cumulative dose had been excreted in feces, 38.3% in urine and 1.8% in expired air. 0.7% of the dose was retained in the body.

f) After 14 days of dosing, whole body autoradiography revealed that most of the radioactivity was in the mandibular lymph node (*Reviewer's comment: The report repeatedly refers to the mandibular lymph node, but sometimes qualifies it as "lumbar" or with other locations; apparently the term is being used to refer to lymph nodes in general and not to that specific lymph node. Successive references in this review will be to "lymph node" only.*), intestinal contents, adrenal gland, liver, spleen and bone marrow.

After 14 consecutive daily doses to male rats, radioactivity levels were determined in tissues at 24 hours after the first, seventh, and fourteenth doses. Those levels increased with successive doses, and plateaued after the seventh dose in plasma, whole blood, lymph node, and white fat. Radioactivity in most tissues 24 hours after the 14th dose was more than four times the radioactivity in the same tissues 24 hours after a single dose. The most marked increases were in spleen, testis, eye, and bone marrow (up to 10-fold increase after 14th dose).

Elimination occurred more slowly from tissues after the 14th dose than after a single dose. At 168 hours after the 14th dose, white fat, small intestine, testis, adrenal, stomach, and spleen had radioactivity levels that were 15-21 times higher than at the same time after a single dose. One hundred days after dosing, significant levels were still present in testis, lymph node, and adrenal glands (13-24% of maximal values). The half-lives in these tissues were 86, 180, and 120 days, respectively. Radioactivity levels in the liver, kidney, spleen and bone marrow were 0.7-9.7% of maximal values at 100 days, with tissue half lives 29-83 days.

g) A single iv dose of 2 mg/kg was given to nursing rats. Peak levels of 0.66 µg-equivalents/ml were detected at 30 minutes in milk. These levels declined with a half-life of 17 hours. At 2 hrs post-administration, radioactivity in milk was eliminated more slowly than in plasma, resulting

in 3.6 and 4 times higher milk concentrations relative to plasma concentrations at 24 and 48 hrs, respectively.

h) After a single iv dose of 2 mg/kg to male rats, plasma concentrations determined by HPLC were 1.91 µg/ml at 5 minutes and 0.08 µg/ml at 6 hours. HPLC revealed the following plasma metabolites: one metabolite peak at a retention time of 3-5 minutes and multiple peaks at retention times of 18-40 min. After 14 consecutive daily doses of 2 mg/kg iv in male rats, the HPLC metabolite profile for plasma was similar to that after a single dose.

The parent compound was not detected in the urine voided by 24 hours after administration. HPLC metabolite peaks in urine were: two peaks with retention times of 3-5 minutes, multiple peaks at 18-35 min, and four peaks at 35-43 minutes. The urinary metabolite profile after a single dose was similar to that after the 14th consecutive daily dose.

After a single 2 mg/kg iv dose to male rats, 12.1% of the administered radioactivity recovered in the feces by 24 hours after dosing was associated with the parent compound. Major HPLC peaks were: multiple peaks with retention times of 20-40 minutes, a single peak at 44 minutes, and another single peak at 54 minutes. Twenty-four hours after the 14th consecutive daily dose, 8.5% of fecal radioactivity was associated with the parent compound. The metabolite profile was similar to that after a single dose except that the peak height at 54 minutes retention time was less.

Thirteen percent of radioactivity after a single dose of 2 mg/kg was recovered in bile (via cannulated bile ducts) by 8 hours post-administration. Major HPLC peaks were: multiple peaks at retention times of 18-32 minutes, 3 peaks at 35-40 minutes, and a single peak at 43 min. The metabolite profile was similar after repeated dosing.

PK parameters:

Plasma half-life: In rats, apparent $t_{1/2}$ = 1.3 hours for first four hours following administration; apparent $t_{1/2}$ = 15 hours from 6-72 hours post-administration. In dogs, $t_{1/2}$ = 1.4-1.7 hours

Absorption: NA (administered parenterally)

Distribution: See above.

Metabolism: See above.

Excretion: In rats, approximately 100% of administered dose cleared within 48 hours, with activity primarily in the urine, but substantial amounts eliminated in the feces. In dogs, approximately 97% of an administered dose was eliminated within 72 hours, with about 2/3 of the activity in the urine and the remainder in the feces.

Other studies: NA

PK/TK summary: The drug substance is administered intravenously, rapidly distributed (but does not cross the blood-brain barrier), extensively metabolized, cleared within 48-72 hours in rats and dogs, and eliminated primarily in the urine, but with a substantial fraction in the feces.

PK/TK conclusions: In rats and dogs, polidocanol is extensively metabolized, cleared within 48-72 hours, and eliminated primarily in the urine.

IV. GENERAL TOXICOLOGY:

1. Study title: Investigation of Aethoxysklerol® on acute toxicity in rats after intravenous injection

Key study findings: When administered IV to rats, the NOEL was reported to be 2 mg/kg and the LD₅₀ was reported to be between 20 and 100 mg/kg.

Study no: 024 TOX 94

Volume # and page #: Reference 32, Volume 20 of 50, pp. 1002-1036.

Conducting laboratory and location: (b) (4)

(b) (4)

Date of study initiation: February 23, 1994 (receipt of animals)

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: polidocanol (Kreussler), batch #30848, purity not provided

Formulation/vehicle: Aethoxysklerol 4% (b) (4)/buffered aqueous solution

Methods (unique aspects):

Dosing:

Species/strain: Wistar rats

#/sex/group or time point (main study): 5/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: not specified

Weight: 191-222 g males; 175-189 g females

Doses in administered units: 2, 20, 100 mg/kg (12, 120, 600 mg/m²), 0.25 ml/100 g body weight (bw). Doses were 0.33, 3.3, and 16.7 times the human dose on a mg/m² basis.

Route, form, volume, and infusion rate: single dose, iv in tail vein, 0.25 ml/100 g bw, 0.05 ml/sec.

Observations and times:

Clinical signs: Observed on the day of dosing three times at 3-4 hour intervals, beginning not more than one hour after dosing, and daily for 14 days after dosing. Note that one male in each of the 20 and 100 mg/kg dose groups was not evaluated due to extravasation of the dose.

Body weights: recorded pre-dosing and at termination

Food consumption: not performed.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Gross pathology: Necropsy performed on all animals.

Organs weighed: not performed.

Histopathology: Only performed on tissues associated with a macroscopic finding at necropsy.

Toxicokinetics: not performed.

Results:

Mortality: All animals in the 100 mg/kg dose group died immediately after injection.

Clinical signs: At 2 mg/kg, no signs were observed. At 20 mg/kg, on the day of dosing, all animals showed reduced activity immediately after injection. Local reactions were noted over time in the tail consisting of discoloration, necrosis, lost tail tips in 3 animals. No local reaction was noted in 2/4 males and 1/5 females. One male and 2 females exhibited local effects only until 6-8 days after administration. One female exhibited convulsions approximately 30 minutes after dosing.

Body weights: At 7 days after administration, decreased body weight was observed in females, and the body weight gain in males was less than that of 2 mg/kg group (not significant). At 14 days, body weight was still depressed in males only.

Food consumption: not performed.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Organ weights: not performed

Gross pathology: No alterations seen at 2 mg/kg. Coloring of lymph nodes was reported in 20 mg/kg animals and considered to be due to storage of hemosiderin and indicative of hemolysis after injection. In 100 mg/kg animals, findings of acute congestion of venous vessels, thymus and pleural hemorrhage, soapy and/or wet peritoneum and livid-brown flesh were noted. One animal had foamy contents in the trachea.

Histopathology: Hemorrhagic lung edema noted in all 100 mg/kg animals. At 20 mg/kg, hyperplasia of lymph nodes was noted and was considered to be related to the local reactions in the animals' tails.

Toxicokinetics: not performed.

Summary of individual study findings: Based on the absence of clinical signs of toxicity and pathology findings, the NOEL was reported to be 2 mg/kg (0.32 times the maximum human dose after normalizing the data on the basis of body surface area). Clinical findings were noted in all mid-dose animals (20 mg/kg, 3.2 times the maximum human dose based on body surface area). Because all animals died in the high dose, and none in the mid-dose, the LD₅₀ was considered to be between 20 and 100 mg/kg (between 3.2 and 16.2 times the maximum human dose based on body surface area).

2. Study title: Investigation of Aethoxysklerol[®] on acute toxicity in rats after subcutaneous injection.

Key study findings: When administered subcutaneously to rats, the NOEL was considered to be 4 mg/kg for systemic toxicity (0.65 times the maximum human dose based on body surface area), and the LD₅₀ was greater than 1000 mg/kg (162 times the maximum human dose based on body surface area). All exposures used in this study induced reactions at the injection site.

Study no: 023 TOX 94

Volume # and page #: Reference 34, Volume 20 of 50, pp. 1100-1140.

Conducting laboratory and location: (b) (4)

Date of study initiation: February 23, 1994 (receipt of animals)

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: polidocanol (Kreussler), batch #30848, purity not provided

Formulation/vehicle: Aethoxysklerol 4% (b) (4)/buffered aqueous solution

Methods:

Dosing:

Species/strain: Wistar rats

#/sex/group or time point (main study): 5/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: not specified

Weight: 190-223 g males; 171-190 g females

Doses in administered units: 4, 200, 1000 mg/kg (24, 1200, and 6000 mg/m²), 2.5 ml/100g. Doses were 0.65, 32, and 162 times the human dose on a mg/m² basis.

Route, form, volume, and infusion rate: single dose, sc, half on left side and half on right side

Observations and times:

Clinical signs: Observed on the day of dosing three times at 3-4 hour intervals, beginning not more than one hour after dosing, and daily for 14 days after dosing.

Body weights: recorded pre-dosing, 7 days post-dosing, and at termination (day 14).

Food consumption: not performed.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Gross pathology: Necropsy performed on all animals.

Organs weighed: not performed.

Histopathology: Only performed on tissues associated with a macroscopic finding at necropsy.

Toxicokinetics: not performed.

Results:

Mortality: At 1000 mg/kg, 1/5 males died at approximately 24 hours after dosing and 2/5 females were found dead on the second day.

Clinical signs: At 4 mg/kg, local reactions were noted in the area of injection consisting of a small amount of hair loss and scabbing in one male and two females. At 200 mg/kg, the skin reactions consisted of severe damage in all animals. At 1000 mg/kg, signs observed in the male animal that died included somnolence, slow movement, inactivity,

dyspnea, cool body surface, tremor, cyanosis. In the females that died, shaggy fur, decreased activity, slowdown of movement, low body surface temperature, decreased skin turgor, and tremor were reported. In surviving animals, observed signs included decreased activity, slowdown of movement, shaggy fur, tremor, low body surface temperature, and decreased skin turgor. Local signs at the injection site were noted and were dose-related in severity.

Body weights: At 7 and at 14 days after administration, body weight was depressed in males and females at this dose, although not statistically significant.

Food consumption: not performed.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Organ weights: not performed

Gross pathology: Scar producing granulation tissue was found at the injection site at all doses, and its intensity was dose-related. In the animals found dead (1000 mg/kg), findings included edematous changes of tissue at the injection site and in the lungs, venous congestion and thymic bleeding.

Histopathology: Slight lymphatic hyperplasia, considered to be secondary to local inflammation at the injection site, was also noted.

Toxicokinetics: Not performed.

Summary of individual study findings: In rats dosed subcutaneously with polidocanol, the NOEL was considered to be 4 mg/kg for systemic toxicity (0.65 times the maximum human dose based on body surface area), and the LD₅₀ was greater than 1000 mg/kg (162 times the maximum human dose based on body surface area). At 200 mg/kg (32 times the maximum human dose based on body surface area) and above, local irritation, lymphatic hyperplasia, and reduced body weights were noted. Mortality was observed only in the high dose animals (1/5 males; 2/5 females). Granulation tissue was found at the injection site at all doses, and its intensity was dose-related. Note that this study did not include animal sacrifice at an early time point (e.g., 48 hours post-injection), so it is unclear what the maximum extent of tissue damage and necrosis may have been.

3. Study title: Investigation of Aethoxysklerol® on acute toxicity in rabbits after intravenous injection.

Key study findings: When administered IV to rabbits, the NOEL was reported as 2 mg/kg (0.65 times the maximum human dose based on body surface area). Because all animals died in the high dose group, the LD₅₀ was considered to be between 20 and 100 mg/kg (6.5 to 32 times the maximum human dose based on body surface area).

Study no: 026 TOX 94

Volume #, and page #: Reference 35, Volume 20 of 50, pp. 1141-1173.

Conducting laboratory and location: (b) (4)

Date of study initiation: March 16, 1994 (receipt of animals)

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: polidocanol (Kreussler), batch #30848, purity not provided

Formulation/vehicle: Aethoxysklerol 4% ((b) (4))/buffered aqueous solution

Methods:

Dosing:

Species/strain: Outbred Russian rabbits

#/sex/group or time point (main study): 5/female/group

Satellite groups used for toxicokinetics or recovery: none

Age: not specified

Weight: 1.7-2.0 kg

Doses in administered units: 2, 20, 100 mg/kg (24, 240, 1200 mg/m²), 2.5 ml/kg bw.

Doses were 0.67, 6.7, and 33 times the maximum human dose on a mg/m² basis.

Route, form, volume, and infusion rate: single dose, iv in ear vein, 2.5 ml/kg, 0.05 ml/sec.

Observations and times:

Clinical signs: Observed on the day of dosing three times at 3-4 hour intervals, beginning not more than one hour after dosing, and daily for 14 days after dosing.

Body weights: recorded pre-dosing, 7 days post-dosing, and at termination (day 14).

Food consumption: not performed.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Gross pathology: Necropsy performed on all animals.

Organs weighed: not performed.

Histopathology: Only performed on tissues associated with a macroscopic finding at necropsy.

Toxicokinetics: not performed.

Results:

Mortality: One animal in the 20 mg/kg dose group died approximately 1 hour after injection. All animals in the 100 mg/kg dose group died immediately after injection.

Clinical signs: At 2 mg/kg, there were no systemic signs. Local inflammation and scabbing were evident at the injection site at 1-3 days post-administration in all animals to some degree.

At 20 mg/kg, local reactions similar to those in the low dose group were observed, but were greater in severity. At the time of injection, all animals vocalized and fell comatose with rapid respiration followed by tonic spasm of back muscles 5 minutes later. In the animal that was found dead at 1 hour, foamy bloody salivation and bloody rectal efflux were recorded. At 3 hours, 1 animal was somnolent with bloody secretion (the site was not specified). At 6 hours, 2 animals had bloody urine.

Body weights: Depression of body weight gain at 20 mg/kg was reported (although not significantly different from the low dose group).

Food consumption: not performed.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Gross pathology: No pathological alterations were noted at 2 mg/kg. Acute lung edema, sometimes hemorrhagic, was reported as the cause of death in the 6 animals that died.

Other findings in those animals included acute venous congestion, bleeding in the thymus into the parenchyma with hyperemia, and bleeding from the nose and bladder.

Hyperemia and foamy/ bloody contents were also found in the trachea, as well as signs of hemolysis, cyanotic mucous membranes, and bloody exudate into the pericardium.

Organs weighed: not performed.

Histopathology: The liver of one high dose animal was described as "brittle"; histological evaluation revealed acute hemostasis and hydropic hepatocytes in the centers of lobules.

This was reported as indicative of shock.

Toxicokinetics: not performed.

Summary of individual study findings: In rabbits dosed IV with polidocanol, the NOEL was reported as 2 mg/kg (0.65 times the maximum human dose based on body surface area). Because all animals died in the high dose group, the LD₅₀ was reported to be between 20 and 100 mg/kg (6.5 to 32 times the maximum human dose based on body surface area). Dose-related increases in severity of local inflammation were reported in all animals. Systemic toxicity such as signs of hemolysis, cyanotic mucous membranes, bloody exudate into the pericardium, convulsions, and reduction in body weight gains were noted in 20 mg/kg animals.

4. Study title: Investigation of Aethoxysklerol[®] on acute toxicity in rabbits after subcutaneous injection.

Key study findings: In rabbits dosed subcutaneously with polidocanol, the NOEL for systemic toxicity was considered to be 4 mg/kg, although an effect on body weight was seen at this dose. The LD₅₀ was considered to be between 200 and 1000 mg/kg.

Study no: 025 TOX 94

Volume #, and page #: Reference 36, Volume 20 of 50, pp. 1174-1208.

Conducting laboratory and location: (b) (4)

Date of study initiation: March 16, 1994 (receipt of animals)

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: polidocanol (Kreussler), batch #30848, purity not provided

Formulation/vehicle: Aethoxysklerol 4% (b) (4)/buffered solution

Methods:

Dosing:

Species/strain: Russian outbred rabbits

#/sex/group or time point (main study): 5/female/group (one high dose animal was found to be a cryptorchid male at necropsy)

Satellite groups used for toxicokinetics or recovery: none

Age: not specified

Weight: 1.7-1.9 kg

Doses in administered units: 4, 200, 1000 mg/kg (44, 2200, and 11,000 mg/m²), 1.3, 67, and 333 times the human dose on a mg/m² basis.

Route, form, volume, and infusion rate: single dose, sc, given in 5 areas on the neck, back, and flanks, 0.25 ml/kg

Observations and times:

Clinical signs: Observed on the day of dosing three times at 3-4 hour intervals, beginning not more than one hour after dosing, and daily for 14 days after dosing.

Body weights: recorded pre-dosing, 7 days post-dosing, and at termination (day 14).

Food consumption: not specified, but results indicate food consumption was monitored at some point during the study.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Gross pathology: Necropsy performed on all animals.

Organs weighed: not performed.

Histopathology: Only performed on tissues associated with a macroscopic finding at necropsy.

Toxicokinetics: not performed.

Results:

Mortality: One animal in the 200 mg/kg group died at approximately 26 hours after dosing. All high dose animals died within 3.5-7.5 hours after dosing.

Clinical signs: At 200 mg/kg, all animals exhibited the following signs from immediately after injection until approximately one day afterwards: tonic spasms, apathy (coma-like) or somnolence, labored respiration, delayed reactions, decreased food consumption, anuria or bloody urine, decreased or no defecation, and the body surface was cool. On the second day after dosing, three of the survivors exhibited slight somnolence and/or decreased food consumption, or bloody urine. From the fourth day onward, local reactions at the injection sites were observed. At 1000 mg/kg, tonic spasms, hyporeflexia, coma, accelerated respiration, cool body surface, dyspnea, salivation, watery ocular discharge, efflux of bloody urine, and redness at the injection sites were observed prior to death.

Body weights: The body weights of animals in the 200 mg/kg dose group were significantly lower than those in the low dose group at 7 and at 14 days after injection.

Food consumption: see clinical signs.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Gross pathology: At 4 mg/kg, the only treatment-related observations were local alterations at injection sites, with indications of old hemorrhage.

At 200 mg/kg, the animal found dead had necrosis of the liver that was attributed to shock, and hemorrhagic edema at the injection sites. In the other animals, the only findings were necrosis and inflammatory changes at the injection sites.

At 1000 mg/kg, the cause of death was identified as acute lung edema. Pathological changes of note were venous congestion, acute hemorrhagic lung edema and hyperemia, bleeding in the thymus, foamy bloody content in the trachea, cyanotic mucous membranes, bloody excretion from the urethra, and reddened mucous membranes in the urinary bladder.

Organs weighed: not performed.

Histopathology: At all doses, there were local injection site reactions consisting of inflammation, edema, and necrosis. These were dose-related in severity and the degree of formation of granulation tissue.

Toxicokinetics: not performed.

Summary of individual study findings: In rabbits dosed subcutaneously with polidocanol, the NOEL for systemic toxicity was considered to be 4 mg/kg (1.3 times the maximum human dose based on body surface area.) However, an effect on body weight was seen at this dose, and therefore this may not be an accurate conclusion. The LD₅₀ was considered to be between 200 and 1000 mg/kg (65-324 times the maximum human dose based on body surface area). Local toxicity at the site of injection was noted in all dose groups, and severity increased with dose.

5. Study title: Single-dose subcutaneous and intravenous toxicity test of polidocanol in rats.

Key study findings: The LD₅₀ for acute subcutaneous administration of polidocanol was 1146.6 mg/kg for male rats and 953.7 mg/kg for females. The LD₅₀ for acute IV administration was 62.6 mg/kg for male rats and 64.4 mg/kg for females.

Study no: (b) (4)-89-RZAC-118

Volume # and page #: Reference 33, Volume 20 of 50, pp. 1037-1099.

Conducting laboratory and location: (b) (4)

(b) (4)

Date of study initiation: October 12, 1989

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: polidocanol, lot #04456

Formulation/vehicle: injectable solution/distilled water

Methods:

Dosing:

Species/strain: Crj:CD rats

#/sex/group or time point (main study): 5/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: 6-weeks at initiation

Weight: 128-144 g males; 113-126 g females

Doses in administered units: 500, 650, 845, 1098, and 1428 mg/kg SC (3000, 3900, 5070, 6588, 8568 mg/m²), 50, 57.5, 66.1, 76, 87.5, 100.6 mg/kg IV (300, 345, 397, 456, 525, 604 mg/m²). Subcutaneous doses were 83-238 times the maximum human dose on a mg/m² basis. Intravenous doses were 8.3-16.8 times the maximum human dose on a mg/m² basis.

Route, form, volume, and infusion rate: single dose, injected either into the subcutaneous tissue of the back or into the tail vein

Observations and times:

Clinical signs: Monitored continuously for the first 3 hours after dosing, then several times in the next two hours, then daily for 14 days after dosing.

Body weights: recorded pre-dosing, and on days 1, 3, 5, 7, and 14 post-treatment

Food consumption: not performed

Ophthalmoscopy: not performed

EKG: not performed

Hematology: not performed

Clinical chemistry: not performed

Urinalysis: not performed

Gross pathology: Necropsy of all study animals.

Organs weighed: not performed.

Histopathology: performed in 2 groups closest to the LD₅₀.

Toxicokinetics: not performed

Results:

Mortality: Deaths occurred from 3-5 hours after dosing until the next morning in the sc group as follows: 3/5 females at 845 mg/kg, 2/5 males and 3/5 females at 1098 mg/kg, and all animals dosed at 1428 mg/kg. Deaths occurred from immediately to 7 minutes after dosing in the iv group as follows: 2/5 males and 2/5 females at 57.5 mg/kg, 4/5 males and 3/5 females at 66.1, 4/5 males and 4/5 females at 76 mg/kg, and all animals dosed at 87.5 and 100.6 mg/kg.

Clinical signs:

for sc dosing: At doses of 650 mg/kg and higher, decreased spontaneous movement from about 25 minutes after dosing, prone, lateral or supine position, ataxia, bradypnea and respiratory depression were observed. In most of the surviving rats, signs resolved by the day after dosing. Local effects consisting of induration at the injection site, hair loss, scratch marks, and scabbing beginning day 2 after dosing were seen.

for iv dosing: At all doses, adoption of supine, lateral or prone position, bradypnea, hematuria, reduced spontaneous movement, tonic convulsions, and opisthotonos (tetanic spasms) were observed from just after dosing. At doses of 87.5 mg/kg and higher, rats died immediately without development of signs other than severe respiratory depression and arrest. The surviving rats returned to normal appearance by 3 hours after dosing. Local effects consisting of reddening of the tail at 50 mg/kg or higher and necrosis distal to injection site were seen on days 4-9 after dosing.

Body weights: Transient body weight loss was noted in the 1098 mg/kg group (SC)

Food consumption: not performed

Ophthalmoscopy: not performed

EKG: not performed

Hematology: not performed

Clinical chemistry: not performed

Urinalysis: not performed

Gross pathology:

for sc dosing: Rats that died had subcutaneous edema and hemorrhage at the injection site, pulmonary edema and congestion, reddish areas in the lungs. In the survivors, hair loss and scabbing at the injection site were seen at doses of 500 mg/kg and above.

for iv dosing: In the rats that died, hematuria and pulmonary edema were evident on gross necropsy. In survivors, necrosis of the injection site or loss of a necrotic tail was noted at 50 mg/kg and higher. Scab formation, acanthosis, granulation tissue in the vein at the injection site were seen at 57.5 mg/kg and higher.

Organs weighed: not performed.

Histopathology:

for sc dosing: Acanthosis and a decrease in skin appendages were seen at doses of 845 and 1098 mg/kg. Dermal ulceration and/or necrosis, subcutaneous granulation tissue and fibrosis, degeneration and calcification of the muscular layer were seen at the injection site at 845 mg/kg. Focal necrosis of the tail vein was seen at 845 and 1098 mg/kg. Focal hemorrhage, edema and cellular infiltration were seen at 1098 mg/kg.

for iv dosing: Histological evaluation revealed slight necrosis of the vein wall at the injection site in animals that died prematurely. At 66.1 mg/kg, epidermal necrosis, perivascular calcification of the muscular layer, cellular infiltrate, abscess formation, granulation tissue, and fibrosis were noted in association with the injection site. Additionally, discoloration and enlargement of the kidneys in the one surviving male at 66.1 mg/kg was histologically described as hyaline droplet degeneration of the renal tubular epithelium and tubular dilatation.

Toxicokinetics: not performed

Summary of individual study findings: The LD₅₀ for acute subcutaneous administration of polidocanol was 1146.6 mg/kg for male rats and 953.7 mg/kg for females (186 and 155 times respectively the maximum human dose based on body surface area). The LD₅₀ for acute iv administration was 62.6 mg/kg for male rats and 64.4 mg/kg for females (approximately 10 times the maximum human dose based on body surface area). Similar signs of toxicity were noted in rats dosed with polidocanol by either IV or SC injection, although most intense and rapid in onset following IV administration.

6. Study title: Single-dose subcutaneous and intravenous toxicity test of polidocanol in mice

Key study findings: The LD₅₀ for subcutaneous administration was considered to be 837 mg/kg in male mice and 792 mg/kg in females. The LD₅₀ for intravenous administration was determined to be 118 mg/kg in male mice and 135 mg/kg in females. Local inflammatory effects were noted following both SC and IV injection, although most intense and rapid in onset following IV administration.

Study no: (b) (4)-89-MZAC-119

Volume #, and page #: Reference 26, Volume 19 of 50, pp. 602-664.

Conducting laboratory and location: (b) (4)

(b) (4)

Date of study initiation: October 12, 1989

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: polidocanol, lot #04456

Formulation/vehicle: injectable solution/distilled water

Methods:

Dosing:

Species/strain: ICR mice

#/sex/group or time point (main study): 5/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: 6 weeks

Weight: 27.1-35.6 g males; 21.8-26.7 g females

Doses in administered units: 500, 650, 845, 1098, and 1428 mg/kg SC (1500, 1950, 2535, 3294, 4284 mg/m²), 75, 94, 117, 147, 183 mg/kg IV (225, 281, 352, 440, 549 mg/m²).

Subcutaneous doses were 41-116 times the human dose on mg/m² basis. Intravenous doses were 6-15 times the human dose on a mg/m² basis.

Route, form, volume, and infusion rate: single dose, injected into the subcutaneous tissue of the back or into the tail

Observations and times:

Clinical signs: monitored continuously for the first 3 hours after dosing, then several times in the next two hours, then daily for 14 days after dosing.

Body weights: recorded pre-dosing and on days 1, 3, 5, 7, and 14 post-dosing

Food consumption: not performed

Ophthalmoscopy: not performed

EKG: not performed

Hematology: not performed

Clinical chemistry: not performed

Urinalysis: not performed

Gross pathology: Necropsy of all study animals.

Organs weighed: not performed.

Histopathology: performed in 2 groups closest to the LD₅₀.

Toxicokinetics: not performed

Results:

Mortality: Deaths occurred from 30 minutes after dosing through the next morning in the SC group as follows: 3/5 males and 4/5 females at 845 mg/kg, and all animals at 1098 and 1428 mg/kg (*Comment: The doses listed in the table for subcutaneous administration were those for the IV group; the above dose distribution for deaths is based on relative doses*). Deaths occurred within the first 9 minutes after dosing in the IV group as

follows: 1/5 males at 93.8 mg/kg, 3/5 males and 2/5 females at 117.2 mg/kg, 4/5 males and 3/5 females at 146.5 mg/kg, and all animals at 183.1 mg/kg.

Clinical signs:

for sc dosing: At all doses, observations included decreased spontaneous movement, supine, prone or lateral position, tonic/clonic convulsions and opisthotonos, bradypnea, and severe respiratory depression. In survivors, signs resolved by the day after administration except for one mouse in the 845 mg/kg group. Injection site effects included induration of the skin, followed by loss of hair, scratch marks, and scabbing. These were completely resolved in only one mouse in the 500 mg/kg group by end of study.

for iv dosing: At all doses, observations included decreased spontaneous movement, supine, prone or lateral position, tonic/clonic convulsions and opisthotonos, and bradypnea. Signs resolved in about 1 hour after dosing in surviving mice. Local effects included reddening of the tail skin and necrosis of the tail in all animals.

Body weights: In animals dosed SC, body weight loss or lack of gain was observed from the day after administration through day 3 (males) or 5-7 (females) after dosing. In animals dosed IV, body weight loss or poor gain was noted through day 3 after dosing in all groups.

Food consumption: not performed

Ophthalmoscopy: not performed

EKG: not performed

Hematology: not performed

Clinical chemistry: not performed

Urinalysis: not performed

Organ weights: not performed

Gross pathology:

for sc dosing: Subcutaneous hemorrhage was noted in all of the mice that died, with or without early leukocyte or macrophage infiltration, focal dermal edema and hemorrhage, and pulmonary congestion (the latter was seen in 1 male and 1 female mouse at 1098 mg/kg; the male also had evidence of focal edema and hemorrhage). In survivors, local effects of hair loss, scabbing, and ulceration at the injection site were seen.

for iv dosing: In the mice that died, pulmonary congestion and edema were noted on gross examination. In survivors, local effects of necrosis and skin loss at the injection site and scabbing were seen.

Histopathology:

for sc dosing: Acanthosis and a decrease in skin appendages at the injection site were noted. Subcutaneous granulation tissue was noted in some. Other items noted at the site included focal cell infiltration, focal fibrosis, and edema with macrophage infiltration, dermal fibrosis, and granulation tissue. In the one surviving female at 845 mg/kg, it was noted that the surface of both kidneys was roughened; this was diagnosed histologically as bilateral chronic interstitial nephritis.

for iv dosing: Perivascular hemorrhage, edema diffuse necrosis and focal acanthosis at the injection site; Also noted were dermal necrosis with abscess formation, dermal ulcers, perivascular granulation tissue and fibrosis, granulation tissue in the tail vein, and cellular infiltration.

Toxicokinetics: not performed

Summary of individual study findings: The LD₅₀ for subcutaneous administration was considered to be 837.1 mg/kg in male mice and 792.3 mg/kg in females (70 and 66 times greater respectively than the maximum human dose based on body surface area). The LD₅₀ for intravenous administration was determined to be 118 mg/kg in male mice and 135 mg/kg in females (10 and 11 times greater respectively than the maximum human dose based on body surface area). Local inflammatory effects were noted in following both SC and IV injection, although most intense and rapid in onset following IV administration. In mice that died after treatment, edema and hemorrhage at the injection site were noted following both SC and IV injection. Dose-related adverse effects on respiration were also noted.

7. Study title: Acute toxicity of polidocanol by intravenous administration to Sprague-Dawley rats.

Key study findings: Rats received a single IV dose of either 2, 20, 50, or 100 mg/kg polidocanol, followed by immediate flushing of the vein with saline. Clinical signs of toxicity reported following 20 or 50 mg/kg polidocanol included reduced motility, ataxia, dyspnea, miosis, and muscular hypotonia. The severity and duration of these effects were greater at 50 mg/kg than at 20 mg/kg. All effects were resolved within 1 hr post-dosing. No significant findings were reported following recovery of 2 weeks. 100 mg/kg was lethal to all animals within 1 min of dosing. No local toxicity was reported in any dose group, probably because the polidocanol dose was followed with 0.5 ml of 0.9% NaCl to prevent vein sclerosis. The LD₅₀ was calculated as 71 mg/kg. The NOEL for systemic toxicity was 2 mg/kg (0.32 times the maximum human dose based on body surface area).

Study no: 11584/98

Volume # and page #: Reference #31, Volume 20 of 50, pp. 953-1001.

Conducting laboratory and location:

(b) (4)

(b) (4)

Date of study initiation: August 14, 1998

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: polidocanol, batch #V/97/7316, purity not provided.

Formulation/vehicle: polidocanol dissolved in 0.9% NaCl

Methods: Each animal received a single dose of polidocanol solution by i.v. dosing into the tail vein. After administration, the needle was flushed with 0.5 ml 0.9% NaCl to prevent sclerosis of the veins at the injection site.

Dosing:

Species/strain: rat /Sprague-Dawley CrI: CD[®]BR

#/sex/group or time point (main study): 5/sex/group

Satellite groups used for toxicokinetics or recovery: all animals were observed for 2-weeks post-dosing.

Age: approximately 6 weeks

Weight: 176-200 g males; 154-182 g females

Doses in administered units: 2, 20, 50, 100 mg/kg

Route, form, volume, and infusion rate: i.v. into the tail vein, polidocanol solution, 20 ml/kg, dose/30 sec.

Observations and times:

Clinical signs: Observations recorded pre-dosing and at 0 (immediately after), 5, 15, 30, and 60 min, and 3, 6, and 24 hr post-dosing. During the recovery period of 2 weeks, animals were observed once daily for mortality, as well as changes in behavior pattern, skin, fur, eyes, mucous membranes, and vital signs that may have been induced by treatment. Local intolerance reactions were also evaluated.

Body weights: recorded pre-dosing and weekly thereafter.

Food consumption: not monitored.

Ophthalmoscopy: not performed

EKG: not performed

Hematology: not performed

Clinical chemistry: not performed

Urinalysis: not performed

Gross pathology: macroscopic examination of all animals at termination, or of premature deaths, and any changes recorded.

Organs weighed: not performed

Histopathology: not performed

Toxicokinetics: not performed

Results:

Mortality: All HD animals died within immediately following dosing. No other unscheduled deaths were noted. The LD₅₀ was calculated as 70.7 mg/kg.

Clinical signs: None noted in 2 mg/kg animals. All 20 mg/kg animals showed signs of miosis, reduced motility, ataxia, dyspnea, and muscular hypotonia immediately after dosing and continuing up to 30 min post-dosing. 50 mg/kg animals had similar signs, but with greater severity. For up to 15 minutes post-dosing, all animals were in an abdominal position and had reduced motility and ataxia up to 1 hr post-dosing. 100 mg/kg animals showed miosis, severe dyspnea and muscular hypotonia immediately after dosing, and died within 1 min post-dosing.

Body weights: no significant differences noted.

Food consumption: N/A

Ophthalmoscopy: N/A

Electrocardiography: N/A

Hematology: N/A

Clinical chemistry: N/A

Urinalysis: N/A

Organ weights: N/A

Gross pathology: No macroscopic findings were noted in any group.

Histopathology: N/A

Toxicokinetics: N/A

Summary of individual study findings: Clinical signs of toxicity reported following a single dose of 20 or 50 mg/kg polidocanol solution (i.v.) in rats included reduced motility, ataxia, dyspnea, miosis, and muscular hypotonia. These effects increased in severity with dose, and the

duration of these effects also increased with dose. All effects were resolved within 1 hr post-dosing. No significant findings were reported following recovery of 2 weeks. 100 mg/kg was lethal to all animals within 1 min of dosing. No local toxicity was reported in any dose group, likely due to following the polidocanol dose with 0.5 ml of 0.9%NaCl to prevent vein sclerosis. The LD₅₀ was calculated as 71 mg/kg. The NOEL for systemic toxicity was 2 mg/kg (0.32 times the maximum human dose based on body surface area).

8. Study title: Repeated dose study (4 weeks) in beagle dogs after i.v. injection of polidocanol.
Note: The animals in this study did NOT receive the test material daily for 28 days, as the title of the study suggests. Please see "methods", below, for the dosing schedule.

Key study findings: The NOEL for systemic toxicity appears to have been 1 mg/kg (0.5 times the maximum human dose based on body surface area), and the NOEL for local effects was 4 mg/kg (2 times the maximum human dose based on body surface area).

Study no: (b) (4)-66.357-1

Volume #, and page #: Reference #39, Volume 22 of 50, pp. 1864-1982.

Conducting laboratory and location: (b) (4) (b) (4) (b) (4)

Date of study initiation: March 10, 1986 (first dose)

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: polidocanol, batch #0445,

Formulation/vehicle: polidocanol dissolved/diluted with 5% alcohol in physiological saline

Methods (unique aspects): As detailed in the table below, animals were dosed intermittently a total of 6 times over a 4-week period.

week #	day 1	day 2	day 3	day 4	day 5	day 6	day 7
1	dose	dose	no dose	dose	no dose		
2	no dose						
3	dose	dose	no dose	dose	no dose		
4	no dose						
5	necropsy		end of study				

Dosing:

Species/strain: Beagle dogs

#/sex/group or time point (main study): 4/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: 4-5 months

Weight: 9.5-12 kg males; 9-13.5 kg females

Doses in administered units: 0, 1, 4, and 16 mg/kg

Route, form, volume, and infusion rate: i.v., polidocanol solution, 1 ml/kg, 6 ml/min.

Observations and times:

Clinical signs: On days of dosing, animals were observed continuously throughout the treatment period. On non-dosing days, twice daily observations of behavior and physical condition were made.

Body weights: recorded weekly.

Food consumption: recorded weekly.

Ophthalmoscopy: performed pre-dosing, and during weeks 2 and 4.

EKG: performed immediately before and 30 min after the first dosing, 30 min after the 3rd dosing, and during weeks 2 and 4.

Hematology: blood samples collected pre-test, and during week 4 by stilet puncture of the ball of the front limb. Blood gas analysis was also performed pre-test, and during weeks 2 and 4.

Clinical chemistry: same as above.

Urinalysis: performed on urine collected pre-test and during week 4.

Gross pathology: A macroscopic observation of the cranial, thoracic, and abdominal cavities was performed on all animals.

Organs weighed: see table.

Histopathology: see table. Performed on control and high dose animals, as well as target organs from all groups.

Toxicokinetics: not performed

Results:

Mortality: none

Clinical signs: In mid- and high-dose animals, retching, vomiting, ataxia, tremor, salivation, and diarrhea were among the signs noted immediately after injection and continuing for up to 30 min post-dosing (up to 60 min in one HD female). Following cessation of these effects, tiredness was noted. Control and low-dose animals showed no signs of toxicity. On non-dosing days, no remarkable signs were noted.

Body weights: no significant differences.

Food consumption: no significant differences.

Ophthalmoscopy: no treatment-related differences. One LD male had two pigmented moles in the fundus region of the left eye during week 2. This was noted as a common finding in dogs. Redness of the conjunctiva and cloudiness of the cornea in the lower part of the bulbus were noted in the left eye of one mid-dose male in week 4. This was attributed to an injury by scratching.

Electrocardiography: HD females had an elongated PQ interval 30 min after the first dosing relative to the pre-dosing recording, although all values were considered within the normal range. No other abnormal findings were noted.

Hematology: no significant differences reported.

Clinical chemistry: Elevated AST and ALP in MD females, although all values were reportedly within the normal range. No other statistically significant findings were reported.

Urinalysis: increased incidences of protein and blood noted in the urine of polidocanol-treated animals.

Organ weights: absolute liver weights decreased in MD males (no change in relative liver weights); absolute spleen weights increased in LD and HD females (increased relative spleen weights in LD only); absolute adrenal weights in HD females (no change in relative adrenal weights).

Gross pathology: no significant findings reported.

Histopathology: obliteration of the blood vessels at the injection site reported in HD animals only.

Toxicokinetics: N/A

Summary of individual study findings: Based on the clinical effects noted in the MD and HD groups, along with the local effects noted in the HD groups, the NOEL for systemic toxicity appears to have been 1 mg/kg (0.5 times the maximum human dose based on body surface area), and the NOEL for local effects was 4 mg/kg (2 times the maximum human dose based on body surface area). The adverse local effects noted in the HD groups (obliteration of the veins at injection site, 8 times the maximum human dose based on body surface area) is an anticipated reaction due to the pharmacological activity of polidocanol. The ophthalmologic findings do not appear to be dose-related as they were isolated incidences that did not show dose-dependent increases. The finding of PQ interval prolongation in the HD females is of questionable significance, given the fact all other parameters (including QRS intervals) were normal. The increased liver and spleen weights are likely incidental, as no dose-related changes were noted. The increased adrenal weights in HD females may have been polidocanol-related, but this is not clear in the absence of concomitant histopathological findings.

9. Study title: Subacute toxicity test of ASK – 010 intravenous doses intermittently for 4 weeks and recovery test by discontinuation of doses for 4 weeks in dogs.

Key study findings: The NOAEL for systemic toxicity was 3 mg/kg (1.5 times the maximum human dose based on body surface area), and the NOAEL for local toxicity was < 3 mg/kg.

Study no.: (b) (4)-87-DVSA-035

Volume #, and page #: Reference #40, Volume 22 of 50, pp. 1983-2151.

Conducting laboratory and location:

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Date of study initiation: November 30, 1988

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: ASK-010, lot #23189, purity not provided

Formulation/vehicle: 1 ml of ASK-010 contains 10 mg polidocanol and 50 mg ethanol (based on controls, presumably q.s. with saline for injection).

Methods (unique aspects): Animals were dosed every other day for 4 weeks, alternating between the right and left cephalic veins, for a total of 14 treatments.

Dosing:

Species/strain: Beagle dogs

#/sex/group or time point (main study): 3/sex/group

Satellite groups used for toxicokinetics or recovery: 2/group for a 4-week recovery period (see table)

Age: 6 months

Weight: 7.5-10 mg males; 5.8-9.6 mg females

Doses in administered units:

test material	polidocanol (mg/kg/day)	volume (ml/kg/day)	#/sex/treatment	#/sex/recovery
saline	0	1.2	3	-
5% EtOH	0	1.2	3	2
ASK-010	3	0.3	3	-
	6	0.6	3	2
	12	1.2	3	2

Route, form, volume, and infusion rate: i.v., polidocanol solution, 5 ml/min

Observations and times:

Clinical signs: monitored daily for general condition and moribundity/mortality. Hearing was also evaluated (response to a buzz) pre-test and at termination of treatment and recovery phases.

Body weights: recorded twice weekly

Food consumption: recorded twice weekly during the treatment phase, and once during the recovery phase.

Ophthalmoscopy: performed in conjunction with each auditory examination.

EKG: performed in conjunction with each auditory examination.

Hematology: blood samples collected pre-test, during weeks 2 and 4 of the treatment phase, and at termination of the recovery phase.

Clinical chemistry: same as above

Urinalysis: performed on urine samples collected pre-test, during weeks 2 and 4 of the treatment phase, and at termination of the recovery phase.

Gross pathology: performed on animals assigned to the treatment phase 2 days after the final dose, and at the end of the recovery phase for assigned animals.

Organs weighed: see table

Histopathology: see table

Toxicokinetics: not performed

Results:

Mortality: none

Clinical signs: Immediately after dosing, nausea and vomiting were noted in all groups of polidocanol-treated animals. In addition, salivation was noted in the MD and HD groups, and trembling, ataxia, and hematuria were noted in the HD animals. The frequency of these effects was dose-dependent. Stool changes (i.e. soft, mucilaginous, and/or bloody) were noted in all groups (including controls), and persisted through the recovery period. Tumefaction at the site of administration was noted in all groups (except for saline controls) and increased in incidence with dose (all MD and HD animals affected), but resolved within 2 weeks into the recovery period. No abnormalities in auditory function were noted.

Body weights: no significant differences.

Food consumption: sporadic decreases in 1/3 MD male, 1/3 of HD males and females during the treatment phase that were not noted in the recovery phase.

Ophthalmoscopy: on abnormalities noted.

Electrocardiography: no abnormalities noted.

Hematology: In HD females, decreased hematocrit and hemoglobin was noted at week 2 and 4. Increased WBCs and decreased lymphocyte ratio was noted in a HD male at week 2. No findings reported for the recovery phase.

Clinical chemistry:

Urinalysis: sporadic findings of protein in the urine of EtOH control and polidocanol-treated animals. Bilirubin and occult blood was noted in HD males. No significant findings were reported from the recovery period, with the exception of protein in the urine of one MD male

Organ weights: Thymus weights were decreased in 1/3 male and female HD animals; reduced prostate weights in 1/3 LD males.

Gross pathology: The following were noted among ASK-010-treated animals and increased in a dose-dependent manner: local hemorrhage and granule tissue formation at the injection site; development of blood vessels for collateral pathway; tumefaction of the axillary lymph node. Thymus atrophy (correlated with reduced weights) in 1/3 male and female; prostate atrophy in 1/3 LD male (correlated with reduced weight). These effects were not noted in recovery animals. No other dose-related macroscopic findings.

Histopathology: Hemorrhage at the injection site and granule tissue formation in 1/3 EtOH control female and all animals in the MD and HD groups. Other local effects (i.e. fibrillation of vein wall, thrombus, edema, and cellular infiltration) were noted in most ASK-010-treated animals and increased in severity with dose. These effects were also noted after the recovery phase.

Toxicokinetics: N/A

Summary of individual study findings: Dose related increases in the incidence of nausea, vomiting, salivation, and stool changes were noted among dogs dosed i.v. with ASK-010 for 4-weeks, and trembling, ataxia, and hematuria were noted in the HD animals. These effects were not reported during a 4-week recovery period (with the exception of stool changes). Reversible hematological effects (i.e. decreased hematocrit and hemoglobin; increased WBCs and decreased lymphocyte ratio) were also noted in HD animals that were not present in recovery animals. Local adverse effects in ASK-010-treated animals included tumefaction (swelling) at the injection site that was histologically associated with hemorrhage, granule tissue formation, edema, and cellular infiltration. Other dose-dependent local effects included fibrillation of the vein wall, thrombus, and development of blood vessels for collateral pathway. These effects were also noted in recovery animals. Based on these findings, it appears that the NOAEL for systemic toxicity was 3 mg/kg (1.6 times the maximum human dose based on body surface area), and the NOAEL for local toxicity was < 3 mg/kg.

10. Study title: Thirteen week intravenous toxicity study of ASK in rats.

Note: The animals in this study were dosed once per week.

Key study findings: The NOEL for systemic toxicity was considered to be 1 mg/kg weekly for 14 doses (0.16 times the maximum human dose based on body surface area). Injection site damage (discoloration, necrosis, scarring, ulceration) was noted that increased in severity with duration. The high dose (9 mg/kg) group had to be discontinued at week 6 due to the severity of these lesions.

Study no: 93208

Volume #, and page #: Reference 38, Volume 22 of 50, pp. 1799-1863.

Conducting laboratory and location:

(b) (4)

Date of study initiation: November 16, 1993

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: Aethoxysklerol 3%, lot # 29548/900, purity not provided

Formulation/vehicle: 3% Aethoxysklerol diluted in ethanolic phosphate buffer solution containing (per 30 ml) 181.6 mg of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 27 mg KH_2PO_4 , and 1.5 ml of anhydrous ethanol.

Methods:

Dosing:

Species/strain: Crj:CD(SD) rats

#/sex/group or time point (main study): 11/sex/group

Satellite groups used for toxicokinetics or recovery: 5/sex/control and high dose groups were observed for a 4 week recovery period

Age: 5 weeks

Weight: 149-178 g males; 115-141 g females

Doses in administered units: vehicle control, 1, 3, 9 mg/kg/week (6, 18, 54 mg/m^2), or 0.17, 0.5, and 1.5 times the maximum human dose on a mg/m^2 basis.

Route, form, volume, and infusion rate: i.v. into the tail vein, 5 ml/kg; a total of 14 doses were given at weekly intervals, 2 ml/min.

Observations and times:

Clinical signs: Observed twice daily for general condition, moribundity and mortality.

Body weights: recorded weekly

Food consumption: recorded weekly

Ophthalmoscopy: performed pre-dosing, during week 5 (control and high dose only), during week 13 (control, low, mid-dose groups), and after completion of the recovery period (control and high dose only).

EKG: not performed

Hematology: blood samples collected from the abdominal aorta at termination and following the end of the recovery period; CBC with differential, plus clotting.

Clinical chemistry: collection times same as above.

Urinalysis: performed during week 13 and during the last week of the recovery period.

Samples collected at 3 and 21 hr.

Gross pathology: Organs and tissues of cranial, thoracic and abdominal cavities were examined from all study animals.

Organs weighed: see table

Histopathology: see table.

Toxicokinetics: not performed

Results:

Mortality: none

Clinical signs: At 9 mg/kg, hematuria developed in almost all animals within two hours of dosing and was considered to be secondary to hemolysis. Hematuria in some of the control rats was explained as being due to hemolysis caused by the ethanol-containing buffer. Red or purple discoloration of the tail was noted after the third dose in some animals and in almost all animals after the fourth dose. After the fifth dose, the tails of almost all animals in this group were discolored, swollen, scarred, necrotic, ulcerated, or lost. ***Because of severe local toxicity in the high dose group, administration was terminated after the sixth dose;*** animals intended for the recovery study were kept for that period and the rest were necropsied at the time of discontinuation of drug. In recovery animals, discoloration, scarring, and necrosis persisted in 4/5 males and 5/5 females.

At 3 mg/kg, hematuria developed within two hours after each dose from fourth onwards in over half of the animals. Local effects at the injection site gradually became similar to those in the high dose group. By the final dose, those effects were as severe as those in the high dose animals after the sixth dose.

At 1 mg/kg, hematuria developed within two hours of dosing from the third dose onwards in over half of the animals. Local effects at the injection site in the tails after the fourteenth dose were approaching the severity of those in the high dose group at necropsy.

Control animals exhibited hematuria within two hours of dosing from the fourth dose onwards in over half of the rats. No effects were seen in the tails. Sporadic skin ulcers were seen in several control and high dose animals.

Body weights: No significant differences

Food consumption: Lower in the high dose males than that in control males from day 28 through the completion of the recovery period and was significantly less than control during the treatment period. This was associated with nonsignificant decrease in body weight and was coincident with severe local effects in the tails of these animals.

Ophthalmoscopy: No abnormalities were noted in any group.

Electrocardiography: N/A

Hematology:

Males at 9 mg/kg had decreased red blood cell (RBC) counts, hemoglobin, hematocrit, and platelet counts, and increased reticulocyte counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and white blood cell (WBC) counts. The differential WBC count revealed low numbers of lymphocytes and an increase in segmented neutrophils. In females at 9 mg/kg, hematologic findings were similar, but less severe. These changes had resolved by the end of the recovery period.

Males at 3 mg/kg had significant decreases in RBC count, hemoglobin, hematocrit, and platelets. They also had increased segmented neutrophils and decreased numbers of lymphocytes on differential WBC count.

Hematologic findings in 3 mg/kg females and 1 mg/kg dose males and females were similar to those in control animals.

Clinical chemistry:

Serum chemistry changes at the end of treatment in 9 mg/kg males and females included significantly decreased triglycerides, phospholipids, and total protein. Albumin was decreased and globulins were increased, resulting in a decreased A/G ratio. Creatine was increased in the males only. After the recovery period, all parameters returned to values similar to those in control animals with the exception of the low A/G ratio in

males. Liver function enzymes (GOT, GPT, alkaline phosphatase) tended to be slightly increased in high dose males (less than two fold), but were not significantly different from control.

In 3 mg/kg males, significantly decreased triglycerides, albumin, and A/G ratio, increased globulins, and increased creatinine were noted. Liver function enzymes (GOT, GPT, alkaline phosphatase) were slightly increased (approximately two fold), but these were also not significantly different from control due to high variability in the mid-dose group. Females at 3 mg/kg did have a significant increase in globulins.

Serum chemistry findings in 1 mg/kg males and females were similar to those in control animals.

Urinalysis: Urinalyses conducted at the end of treatment and at the end of the recovery period were similar in treated groups and controls.

Organ weights: HD males after treatment had a lower final body weight (as a result, some relative organ weights were somewhat higher than control), higher relative brain weight, higher absolute and relative thymus weights, and lower absolute thyroid weight. After recovery, HD males had a lower absolute thymus weight and higher relative brain and lung weights. These were slight and considered to be incidental. MD males had a lower relative kidney weight. This value was stated to be within the range of background and was considered incidental.

Gross pathology: In the mid dose group, one female had black spots on the gastric mucosa (also seen in one female in the control group). At the low dose, deformity of the caudate lobe of the liver was seen in one male (histologically described as tissue anomaly), one male had black spots in the posterior lobe of the lung (old hemorrhagic foci), one male had enlargement of the submandibular lymph node (lymphadenitis), and one female had multiple renal cysts.

After the recovery period, all high dose animals had tail vein fibrosis, thrombosis with recanalization, and/or tail vein wall damage. Perivascular inflammation and fibrosis were persistent, although less severe than at the time of termination of treatment.

Histopathology: Findings included signs of extramedullary hematopoiesis and brown pigmentation in the spleen, seen in all animals and persisting after the recovery period in high dose and control animals. At the end of treatment, fresh, organized or fibrous thrombi were found in the tail vein in all polidocanol-treated groups. Necrosis, fibrosis, and fibrous hyperplasia were also seen in the walls of the tail vein. In the surrounding tissue, severe hemorrhage, inflammatory changes and fibrosis were seen. Skin necrosis and ulceration were observed at doses of 3 mg/kg and higher. The severity and incidence of all injection site changes was dose-dependent. The only injection site change seen in control animals was perivascular hemorrhage, present only in some animals.

Toxicokinetics: N/A

Summary of individual study findings: In rats dosed i.v. with Aethoxysklerol, injection site damage (discoloration, necrosis, scarring, ulceration) was noted that increased in severity with duration. The high dose (9 mg/kg) group had to be discontinued due to the severity of these lesions. Similar to previous studies, the histological findings associated with this damage was attributed to the pharmacologic effect of polidocanol. Changes in RBC parameters were attributed to hemolysis after injection of the test material. The increases in WBC and alterations in plasma proteins were thought to be due to an inflammatory response at the injection site. Creatinine changes in mid- and high dose males were reportedly within background levels and

not clinically significant as were the lipid alterations in the two higher dose groups. In addition, these effects were reversible upon withdrawal of drug treatment. However, when considering these changes along with borderline increases in liver function enzymes, it is possible that there is some indication of the liver as a target organ of toxicity at higher doses, although concomitant histopathology was not reported. The NOEL for systemic toxicity was considered to be 1 mg/kg weekly for 14 doses (0.17 times the maximum human dose based on body surface area).

11. Study title: Thirteen week intravenous toxicity study of ASK in dogs.

Note: The animals in this study were dosed every other day.

Key study findings: The NOEL for systemic toxicity was considered to be 3 mg/kg when administered IV every other day for 13 weeks (1.6 times the maximum human dose based on body surface area). Hematuria and reduced RBC count, hemoglobin, and hematocrit were noted in high dose (9 mg/kg) animals; these findings were apparently secondary to treatment-related hemolysis. Local toxicity such as perivenous fibrosis was greater in all treated groups than in controls. In high dose animals this local damage at the injection site persisted through the 28-day recovery period.

Study no: 93209

Volume #, and page #: Reference 41, Volume 23 of 50, pp. 2152-2276.

Conducting laboratory and location:

(b) (4)

Date of study initiation: November 16, 1993

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: Aethoxysklerol 3%, lot #29548/900, purity not provided.

Formulation/vehicle: 3% Aethoxysklerol diluted in ethanolic phosphate buffer solution containing (per 30 ml) 181.6 mg of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 27 mg KH_2PO_4 , and 1.5 ml of anhydrous ethanol.

Methods:

Dosing:

Species/strain: Beagle dogs

#/sex/group or time point (main study): 3/sex/group

Satellite groups used for toxicokinetics or recovery: 2/sex/control and high dose groups for a 28-day recovery period

Age: approximately 6 months

Weight: 10.9-13.0 kg males; 9.7-11.6 kg females

Doses in administered units: vehicle, 1, 3, 9 mg/kg (20, 60, 180 mg/m²). Doses were 0.5, 1.6, and 4.9 times the human dose on a mg/m² basis.

Route, form, volume, and infusion rate: intravenous alternately into cephalic or saphenous veins, every other day for 13 weeks for a total of 46 doses in 91 days, 1 ml/kg, 5 ml/min

Observations and times:

Clinical signs: Observed twice daily during the treatment period and once daily during the recovery period for general condition and viability.

Body weights: recorded weekly

Food consumption: recorded weekly

Ophthalmoscopy: performed 1-week pre-dosing, and after 6 and 13 weeks of administration, as well as 4 days prior to and at the end of the recovery period.

EKG: performed 1-week pre-dosing, and after 6 and 13 weeks of administration, as well as 4 days prior to and at the end of the recovery period.

Hematology: performed 1-week pre-dosing, and after 6 and 13 weeks of administration, as well as 1 and 3 days prior to the end of the recovery period. Blood samples were collected from the jugular vein of fasted animals

Clinical chemistry: same as above.

Urinalysis: performed 1-week pre-dosing, and after 6 and 13 weeks of administration, as well as 2 and 4 days prior to and at the end of the recovery period. 3 hr and 21 hr pooled urine samples were used for analysis.

Gross pathology: Organs and tissues of cranial, thoracic and abdominal cavities were examined from all study animals.

Organs weighed: see table

Histopathology: see table.

Toxicokinetics: not performed

Results:

Mortality: none

Clinical signs: Transient salivation was seen during or immediately after dosing in some animals in all treated groups. Transient emesis was seen in some males in the mid- and high dose groups and in females in all treated groups. Overall, these signs were dose-dependent, infrequent at 3 mg/kg, and did not occur in the second half of the treatment period in 3 mg/kg females. At the low dose (1 mg/kg) one female exhibited emesis on one day only. Hematuria was seen in the high dose group in all animals from day 21 onward and was presumed to be due to hemolysis. Local effects included venous sclerosis and perivascular swelling in all treated groups. One high dose male had laceration, ulceration, and scabbing at the injection site(s). Other sporadic signs (decreased food consumption, vomiting, soft, mushy, mucous or watery stools, decreased fecal output, and interdigital swelling) occurred in all groups, including the control group. Signs of local toxicity, interdigital swelling, and soft/watery stools persisted during the recovery period in high dose animals.

Body weights: no significant differences.

Food consumption: no significant differences.

Ophthalmoscopy: no abnormalities noted.

Electrocardiography: no abnormalities noted.

Hematology: In males at 9 mg/kg, hemolysis-related decreases in RBC count, hemoglobin, and hematocrit were seen. Decreased hemoglobin was seen in mid-dose males. These changes were small and were noted after six weeks of dosing. At thirteen weeks, the differences in RBC parameters were no longer statistically significant. No hematological abnormalities were noted after recovery.

Clinical chemistry: At six weeks, high dose males had lower serum sodium concentrations than did controls. At thirteen weeks, serum sodium, α 1 (not significant) and α 3-globulin concentrations were low in high dose males. At thirteen weeks, low dose females had higher inorganic phosphate and calcium concentrations than controls. No abnormalities were noted after recovery

Urinalysis: Results of urinalysis included crystalluria in mid-dose males at 13 weeks. No occult blood was noted in urine at that time, even in animals exhibiting hematuria subsequent to dosing.

Organ weights: Decreased prostate weights in high dose males.

Gross pathology: Injection site effects in treated animals consisted of venous thickening, sclerosis, luminal narrowing, duplication of vein walls, development of collaterals, and increased perivenous connective tissue. In almost all animals, including controls, perivenous edema, yellowing, and signs of hemorrhage were seen. Axillary and popliteal lymph nodes were enlarged in two males and one female at 9 mg/kg, one female at 3 mg/kg, and one male and one female at 1 mg/kg. Many of these signs persisted in high dose animals after recovery. Other incidental findings did not appear to be treatment-related.

Histopathology: Histological findings were related to local irritation at injection sites and regional lymph nodes. Fibrous hypertrophy of the venous intima was seen in treated animals; the severity of this lesion was dose-related. Organization and fibrosis of thrombi and vein wall fibrosis were also seen. In some mid- and high dose animals, fresh thrombus formation in some treated veins was seen and was associated with vessel wall necrosis. Needle tracks, focal necrosis, and granulation of vein walls were identified in the control and two lower dose groups, but were obscured by more severe tissue reaction in the high dose group. Perivenous fibrosis was greater in all treated groups as compared to controls. There was a similar incidence of hemorrhagic and inflammatory reactions in all groups. The enlarged lymph nodes identified on gross necropsy were histologically described as exhibiting reactive lymphoid hyperplasia. Minimal to mild reactive lymphoid hyperplasia without macroscopic changes in the axillary and popliteal lymph nodes was also identified in 1-2 animals in each group, including control.

Incidental findings limited to high dose animals included: 1) cardiac abnormalities - one male with minimal focal coronary arteritis, slight hemangiectasis of and a nodule on the right AV valve, which was associated with brown pigmentation, calcification and fibrosis; one female with slight hemangiectasis in one AV valve; and 2) thyroid abnormalities - minimal to mild parafollicular cell hyperplasia in two males and one female.

After the recovery period, local signs persisted in the high dose group. Fibrotic reactions of the vein and perivascular tissues were present, some with minimal cellular infiltration. Incidental findings limited to high dose animals at this time point included small nodules in the spleen of one female and focal capsular thickening in spleen of another female, mild hemangiectasis in an AV valve in one male, and liver hemangioendothelioma in one male. Additionally, minimal thyroid parafollicular cell hyperplasia was evident in one high dose male and in one control male.

Summary of individual study findings: The NOEL for systemic toxicity was considered to be 3 mg/kg q.o.d. for 13 weeks (1.6 times the maximum human dose based on body surface area). Transient salivation and emesis were noted in males ≥ 3 mg/kg and all treated female groups immediately after dosing, but these symptoms were reversible and did not increase in severity during the study. Hematuria was noted in high dose animals, which was considered associated with treatment-related hemolysis. The reductions in hematological parameters noted in high dose animals were also likely associated with hemolysis. Local toxicity such as perivenous fibrosis was greater in all treated groups as compared to controls. This local damage at the injection site was noted at ≥ 3 mg/kg to persist through the 28-day recovery period. As previously noted, these effects may be attributed to the pharmacologic effect of polidocanol.

12. Study title: 7-day subchronic toxicity study of polidocanol by intravenous administration to Sprague-Dawley rats (Comparison of polidocanol from three different manufacturers).

Key study findings: This study was conducted in an attempt to compare the toxicity profiles of polidocanol obtained from three different suppliers. When administered IV daily for 7 days at a dose of 14 mg/kg, polidocanol (regardless of supplier) was associated with local inflammation at the injection site and effects on the liver (increased liver weight, peripheral fatty infiltration, and vacuolization of hepatocytes). The toxicities associated with polidocanol from three different suppliers were comparable.

Study no: 11128/1/98

Volume #, and page #: Reference 37, Volumes 21 & 22 of 50, pp. 1209-1798

Conducting laboratory and location: (b) (4)

Date of study initiation: October 29, 1998

GLP compliance: yes (x) no ()

QA report: yes (x) no ()

Drug, lot #, and % purity: see table

Formulation/vehicle: aqueous solution, 0.9% NaCl

Methods (unique aspects): animals were dosed daily for 7 days by the tail vein. After test substance administration, the needle was flushed with 1 ml 0.9% NaCl solution per animal to prevent possible sclerosis of the veins at the injection site.

Dosing:

Species/strain: rat/Sprague-Dawley Crl:CD®BR

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

Satellite groups used for toxicokinetics or recovery: none

Age: males 22 days, females 39 days

Weight: 160-172 g (males and females)

Doses in administered units:

group	treatment	Max HED multiple ¹	# per sex	polidocanol batch #
1 (control)	30 ml/kg 0.9% NaCl solution	NA	10	-
2	14 mg/kg reference #1*	2.3	10	04472/172

3	14 mg/kg reference #2**	2.3	10	04479/1140-96
4	2 mg/kg polidocanol***	0.33	10	V/97/7316
5	4 mg/kg polidocanol***	0.33	10	V/97/7316
6	14 mg/kg polidocanol***	2.3	10	V/97/7316

¹Maximum human equivalent dose (HED) based on body surface area using the highest concentration of Aethoxysklerol (3.0%).

*polidocanol supplied by (b) (4)

**polidocanol supplied by (b) (4)

***polidocanol supplied by Kreussler GmbH & Co. (used in to-be-marketed formulation)
Route, form, volume, and infusion rate: i.v. injection (tail vein), bolus solution, 30 ml/kg, approximately 2 min.

Observations and times:

Clinical signs: Once daily for behavioral changes, mortality, or moribundity. Also examined immediately post-dosing for treatment reactions.

Body weights: recorded on day 1 pre-dosing and on day 8 (termination)

Food consumption: recorded as total food given minus food remaining after 1 week.

Ophthalmoscopy: examination on day 1 pre-dosing and on day 8 (termination).

EKG: not performed.

Hematology: blood samples withdrawn at termination

Clinical chemistry: blood samples withdrawn at termination

Urinalysis: urine collected for 16 hr prior to termination.

Gross pathology: All groups necropsied and examined gross lesions/abnormalities.

Organs weighed: see table.

Histopathology: see table. Only performed on G1-3, & 6; target organs (liver and injection site) for all groups.

Toxicokinetics: not performed

Results:

Mortality: none

Clinical signs: Swelling and discoloration of the tail in G2, 3, and 6; no behavioral changes noted; all groups had normally formed feces.

Body weights: no significant differences.

Food consumption: no significant differences.

Ophthalmoscopy: no significant findings.

Electrocardiography: N/A

Hematology: G3 and G6 had decreased hemoglobin (M,F), RBCs (M G3, F G6), hematocrit (M G3, MF G6), and G6 (M,F) increased WBCs relative to controls.

Clinical chemistry: G3 and G6 males had increased ALT, ALP, and AST, and G6 males had increased bilirubin relative to control.

Urinalysis: no significant differences.

Organ weights: increased liver weight in G2, G3 and G6 (only significant in G6 females relative to control).

Gross pathology: Black discolored tail tip in G3 and G6.

Histopathology: Perivascular hemorrhage, lymphohistiocytic infiltration, fibrin, and granulation tissue in G2-6 (most pronounced in G3 and 6, with the aforementioned

effects in the subcutis of the entire tail, accompanied by moderate to severe edema). Peripheral fatty infiltration and vacuolization of hepatocytes noted in G2 (females only), 3 and 6, with the greatest incidence noted in G3 and G6.
Toxicokinetics: N/A

Summary of individual study findings: Intravenous injection of 14 mg/kg polidocanol in 0.9% NaCl, regardless of the supplier of polidocanol, was associated with local inflammation at the injection site, along with macroscopic (increased liver weight) and microscopic (peripheral fatty infiltration and vacuolization of hepatocytes) liver effects. The hematological and clinical chemistry findings may be due to the inflammatory effects noted. However, although statistically significant, the biological significance of these findings is unclear given their minor proportion relative to control (less than 2-fold changes). Therefore, it is apparent that the toxicities associated with polidocanol are comparable among the 3 suppliers.

Other nonclinical toxicology studies:

1. *Extravascular effects of sclerosants in rabbit skin: A clinical and histologic examination.* Goldman et al., 1986, *J. Dermatol. Surg. Oncol.* 12:1085-1088 (Reference #43). Studied the local toxicity of Aethoxysklerol 0.25%, 0.5%, and 1.0%, as well as Sotradecol 0.5% and hypertonic saline 23.4% following intradermal injection. This study was performed in rabbits (n=3) in which each rabbit was injected intradermally on the back with the above solutions along with vehicle controls 5% EtOH and normal saline (i.e. 7 injection sites per animal). At 1, 2, and 5-days post dosing, biopsies of the treatment sites of one rabbit per time point were taken for histopathology. All sclerosant treatment sites had significantly increased erythema scores relative to controls that persisted up to 5 days post-treatment. Necrosis was reported in 0.5% Aethoxysklerol, 0.5% Sotradecol, and 23.4% hypertonic saline. The authors attribute the finding in 0.5% Aethoxysklerol to damage secondary to shaving the animal. Histological examination revealed no remarkable changes in 0.25% and 1.0% Aethoxysklerol, but the 0.5% Aethoxysklerol site had superficial edema and focal RBC extravasation on days 1 and 2. Histology of the Sotradecol-treated sites revealed a lichenoid mixed cellular infiltrate at day 2, also attributed to shaving. Granulocytic inflammation with focal dermal necrosis, extravasated RBCs, and purulent crust were reported at day 5. Hypertonic saline treated sites had lichenoid mixed cellular infiltrate at day 1, in addition to extravasated RBCs and dermal edema. Epidermal and dermal necrosis was reported at day 2, and epidermal, mid-dermis, and adnexal structure necrosis at day 5. The authors conclude that Aethoxysklerol up to 1.0% is less likely to result in necrosis following intradermal injection than either hypertonic saline or Sotradecol. Note that: 1) the formulations of the test materials, other than concentration, were not indicated, and it is therefore unclear how the injected Aethoxysklerol solutions compared to the drug products proposed for marketing under NDA 21-201; and 2) the highest concentration of Aethoxysklerol tested was 1%. Therefore, it is possible that the data described in this study underestimated the potential of the drug product to induce local toxicity if extravasation was to occur.

Toxicology summary:

Acute toxicology:

Species	Route of Admin.	Approx. LD ₅₀ (mg/kg)	NOEL (mg/kg)
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Mouse	IV	118	NA
Mouse	SC	800	NA
Rat	IV	71	2
Rat	SC	>1000	NA
Rabbit	IV	20-100	2
Rabbit	SC	200-1000	4

The primary adverse finding in the acute toxicology studies (other than mortality) was irritation and necrosis in the vicinity of the injection site, which was undoubtedly related to the desired clinical actions of the material (destruction of endothelium leading to replacement of a vessel with connective tissue). As one might expect, the local reaction was intensified (but similar) when the material was injected subcutaneously, since the drug substance was cleared from the site more slowly.

Observations made in repeat-dose toxicology studies largely coincided with observations from acute studies, no doubt at least in part because the repeat-dose studies conducted with polidocanol involved intermittent dosing (usually once or twice per week), rather than daily dosing. This was deemed necessary due to the toxicity of polidocanol at exposures similar to the proposed clinical exposure. Intermittent dosing in repeat-dose studies is acceptable because the proposed clinical use would not involve repeated dosing on a daily basis.

In a repeat-dose toxicology study in which rats were injected with the drug product intravenously at intervals of one week for 13 weeks at dosages of 1, 3, or 9 mg/kg polidocanol (approximately 0.17, 0.5, and 1.5 times the proposed clinical dose, respectively), injection site damage (discoloration, necrosis, scarring, ulceration) was noted that increased in severity with duration. The high dose (9 mg/kg) group had to be discontinued after the sixth dose due to the severity of these lesions. The histological findings associated with this damage were attributed to the pharmacologic effect of polidocanol. Changes in RBC parameters (decreased red blood cell count, hemoglobin, and hematocrit) were attributed to hemolysis after injection of the test material. Increased white blood cell count and alterations in plasma proteins were thought to be due to an inflammatory response at the injection site. The NOEL for systemic toxicity was considered to be 1 mg/kg weekly for 14 doses (0.17 times the maximum human dose based on body surface area).

In a repeat-dose toxicology study in which dogs were injected with the drug product intravenously every other day for 13 weeks at dosages of 1, 3, or 9 mg/kg polidocanol (approximately 0.5, 1.6, and 4.9 times the proposed clinical dose, respectively), the NOEL for systemic toxicity was considered to be 3 mg/kg. A NOEL for local effects was not observed. Transient salivation and emesis were noted in males ≥ 3 mg/kg and all treated female groups immediately after dosing, but these symptoms were reversible and did not increase in severity during the study. Hematuria was noted in high dose animals, which was considered associated with treatment-related hemolysis. The reductions in hematological parameters noted in high dose animals were also likely associated with hemolysis. Local toxicity such as perivenous fibrosis was greater in all treated groups as compared to controls. This local damage at the injection site was noted at ≥ 3 mg/kg to persist through the 28-day recovery period. As previously noted, these effects may be attributed to the pharmacologic effect of polidocanol.

Toxicology conclusions: The primary effects noted in toxicology studies involved damage at or near the injection site, including discoloration, necrosis, scarring, and ulceration. The effects may be attributed to the pharmacologic effect of polidocanol, which acts by damaging endothelium near the injection site when administered intravenously. Systemic effects following repeated intravenous dosing included minor hemolysis, which was probably also the result of the pharmacological actions of the drug substance, which acts to disrupt cell membranes. Substantial multiples of the clinical dose could not be administered in repeat-dose toxicology studies due to dose-limiting local reactions at the injection site.

Histopathology Inventory for NDA #

Study	(b) (4) - 66.357-1 (No. 8 in this review)	(b) (4) -87- DVSA- 035 (No. 9 in this review)	93208 (No. 10 in this review)	93209 (No. 11 in this review)	11128/1 /98 (No. 12 in this review)
Species	dog	dog	rat	dog	rat
Adrenals	X*		X*	X*	X*
Aorta	X	X	X	X	C
Bone Marrow smear					X
Bone (femur)		X	X	X	X
Brain	X*	X*	X*	X*	X*
Cecum	X				C
Cervix		X			X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	C	X*		X	X
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian tube					
Gall bladder				X	
Gross lesions	X				X
Harderian gland			X	X	X
Heart	X*	X*	X*	X*	X*
Ileum	X	X			X
Injection site	X		X	X	X
Jejunum	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*
Lacrimal gland					
Larynx					X
Liver	X*	X*	X*	X*	X*
Lungs	X	X*	X*	X*	X*
Lymph nodes, axillary/politeal		X		X	X
Lymph nodes mandibular	X	X		X	
Lymph nodes, mesenteric		X	X	X	X
Mammary Gland	C	X	X	X	X
Nasal cavity					
Optic nerves		X	X	X	X
Ovaries	X*	X*	X*	X*	X*

Pancreas	X	X	X	X	X
Parathyroid		X*	X*	X*	X*
Peripheral nerve					
Pharynx					C
Pituitary	X	X*	X*	X*	X*
Prostate	C	X*	X*	X*	X
Rectum		X			C
Salivary gland	X	X*	X*		X
Sciatic nerve	C				X
Seminal vesicles	C		X*		C
Skeletal muscle	C				C
Skin	X	X	X	X	C
Spinal cord	X	X	X	XX	X
Spleen	X*	X*	X*	X*	X*
Sternum	X	X	X	X	
Stomach	X	X	X	X	X
Testes	X*	X*	X*	X*	X*
Thymus	X	X*	X*	X*	X*
Thyroid	X	X*	X*	X*	X*
Tongue		X	X	X	C
Trachea	C	X	X	X	X
Urinary bladder	X		X	X	X
Uterus	C		X*	X*	X
Vagina		X	X	X	C
Zymbal gland					
Standard List					

X, histopathology performed

C, tissue collected and retained

*, organ weight obtained

V. GENETIC TOXICOLOGY:

1. Study title: Micronucleus test of Polidocanol Kreussler in bone marrow cells of the NMRI mouse by intravenous administration.

Key findings: Based on the conditions of this assay, Polidocanol (3, 9, or 27 mg/kg) did not result in an increased incidence of micronucleated polychromatic erythrocytes relative to control at 24 or 48 hr. The positive control material (cyclophosphamide, 27 mg/kg, i.p.) did yield a significant increase of PCE (7.8 per 1000 PCEs at 24 hr).

Study no: 16223/02

Study type (if not reflected in title): mouse micronucleus assay

Volume #, and page #: Reference #59, Volume 26 of 50, pp. 3926-3968.

Conducting laboratory and location:

(b) (4)

Date of study initiation: November 8, 2002**GLP compliance:** yes (x) no ()**QA reports:** yes (x) no ()**Drug, lot #, and % purity:** polidocanol, batch #PD-2185, receipt #25903, 98.9% pure (HPLC)**Formulation/vehicle:** white solid diluted in 0.9% NaCl for injection**Methods:**

Strains/species/cell line: Crl:NMRI mice

Basis of dose selection: Doses for the pivotal study were selected based on a previous acute toxicity study in rats in which intolerance reactions (reduced motility, ataxia, reduced muscle tone, and dyspnea) were first noted at 20 mg/kg. Note that the acute toxicity study was performed in rats while the pivotal genotoxicity assay was performed in mice. However, as discussed below, a comparable MTD does appear to have been reached and therefore this cross-species extrapolation appears to be acceptable.

Range finding studies: see above

Test agent stability: not provided

Metabolic activation system: N/A

Controls:

Vehicle: 0.9% NaCl solution, i.v. (tail vein)

Negative controls: 0.9% NaCl solution, i.v. (tail vein)

Positive controls: cyclophosphamide, 27 mg/kg, i.p.

Comments: both polidocanol and cyclophosphamide were diluted in 0.9% NaCl

Exposure conditions:

Incubation and sampling times: see table

Doses used in definitive study: see table

group	compound	dose (mg/kg)	Sampling time (hr)	# animals/sex
1	0.9% NaCl	-	24	5
2	polidocanol	3	24	5
3	polidocanol	9	24	5
4	polidocanol	27	24	5
5	cyclophosphamide	27	24	5
6	0.9% NaCl	-	48	5
7	polidocanol	27	48	5

Study design: At each sampling time point, mice were sacrificed and bone marrow was flushed out of the femur with calf serum, from which a smear was prepared.

Slides were air dried, fixed in MeOH for 5 min, and stained with H&E.

Analysis:

No. of replicates/Counting method: For each animal, 2000 polychromatic erythrocytes (PCE) were scored for micronuclei incidence and the ratio of PCE to normochromatic erythrocytes (NCE) was also determined by counting a total of 2000 erythrocytes. The individual and group mean ratios of PCE/NME was calculated as

well as the individual and group mean frequencies of micronucleated PCE (MN-PCE) per 1000 PCE. All values were compared to historical controls in addition to a comparison of treated vs. vehicle/negative controls.

Criteria for positive results: A positive assay resulted if:

- 1) A statistically significant increase in the frequency of MN-PCE occurred for at least one dose at one sacrifice time.
- 2) The frequency of MN-PCE exceeded the historical control range.
- 3) Corroborating evidence was obtained, for example, increased but statistically insignificant frequencies of MN-PCE at other doses or sacrifice times, or dose response profiles.

Summary of individual study findings:

Study validity: The assay was considered valid based on the following sponsor-defined criteria:

- 1) The heterogeneity χ^2 test provided evidence of acceptable variability between animals within a group.
- 2) The incidence of MN-PCE in the vehicle control groups fell within or close to the historical control vehicle control range.
- 3) At least 7 animals (sexes combined) out of each group were available for analysis.
- 4) The positive control chemical induced clear and statistically significant increases in the frequencies of MN-PCE.

Study outcome: No clinical signs of toxicity were reported in Groups 1-3 or 5-6, while Groups 4 and 7 exhibited ataxia and dyspnea, as well as slightly reduced motility and muscle tone, between 0-5 minutes following injection. Note that all of these signs were also seen in the 20 mg/kg group in the rat acute toxicity study, and therefore a comparable MTD appears to have been reached.

2a. Study title: Mutagenicity test of polidocanol (*Reverse mutation assay*).

Reviewer's note: this report combines two separate studies, a reverse mutation test in E. coli and chromosomal aberration assay in Chinese hamster lung fibroblasts. To avoid confusion, these studies have been separated in this review.

Key findings: No positive responses were observed in any tester strain dosed with polidocanol in the presence or absence of S9. Toxicity was noted in the presence of S9 at ≥ 625 $\mu\text{g}/\text{plate}$, and in the absence of S9 at ≥ 1250 $\mu\text{g}/\text{plate}$. Positive controls showed a clear positive response (26-37 fold increase in mean revertants above vehicle controls). Therefore, this appears to have been a valid assay.

Study no: ML-326A

Study type (if not reflected in title): Reverse mutation test in *E. coli*

Volume #, and page #: Reference #58, Volume 26 of 50, pp. 3888-3925.

Conducting laboratory and location:

(b) (4)

Date of study initiation: November 30, 1987

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug, lot #, and % purity: polidocanol, lot #04454, purity not provided

Formulation/vehicle: polidocanol dissolved in DMSO

Methods:

Strains/species/cell line: *E. coli*/WP2 *uvrA*

Dose selection criteria:

Basis of dose selection: preliminary toxicity test incorporating the maximum feasible concentration (5000 µg/plate).

Range finding studies: 9.77 to 5000 µg/plate were dosed (n=2 plates/dose). Toxicity was noted at ≥ 625 µg/plate, but was not complete even at the maximum dose. No significant difference in the mean revertants were noted in any polidocanol dose.

Test agent stability: stable for up to 1 month

Metabolic activation system: S9 fraction obtained from livers of rats induced with phenobarbital and 5,6-benzoflavone.

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG, 2 µg/plate, -S9)
2-aminoanthracene (2AA, 20 µg/plate, +S9)

Comments: Nutrient broth containing 0.5% NaCl used for pre-incubation. Vogel-Bonner minimum glucose agar medium used for the lower layer, and top agar supplemented with tryptophan and 0.5% NaCl was used for the upper layer.

Exposure conditions:

Incubation and sampling times: see study design.

Doses used in definitive study: 39.1 to 5000 µg/plate

Study design: Using the preincubation method, test materials (n=3 plates/dose) were added in the presence or absence of S9 to a cell suspension for 20 min. Top agar was then added and overlaid onto the lower agar layer for 48 hr incubation at 37°C.

Colony growth inhibition was evaluated as a measure of toxicity.

Analysis:

No. of replicates: 3 plates/dose

Counting method:

Criteria for positive results: A positive response was indicated if a 2-fold increase in mean revertants/plate relative to vehicle controls, and must demonstrate a dose-dependent increase in response.

Summary of individual study findings:

Study validity: see criteria for a positive response.

Study outcome: No positive responses were observed in any tester strain dosed with polidocanol in the presence or absence of S9. Toxicity was noted in the presence of S9 at ≥ 625 µg/plate, and in the absence of S9 at ≥1250 µg/plate. Positive controls showed a

clear positive response (26-37 fold increase in mean revertants above vehicle controls). Therefore, this appears to have been a valid assay.

2b. Study title: Mutagenicity test of polidocanol (*Chromosomal aberration test*).

Reviewer's note: this report combines two separate studies, a reverse mutation test in E. coli and chromosomal aberration assay in Chinese hamster lung fibroblasts. To avoid confusion, these studies have been separated in this review.

Key findings: Polidocanol appears to be positive for numerical chromosomal aberrations in newborn Chinese hamster lung fibroblasts in the absence of metabolic activation.

Study no: ML-326A

Study type (if not reflected in title): *in vitro* chromosomal aberration test

Volume #, and page #: Reference #58, Volume 26 of 50, pp. 3888-3925.

Conducting laboratory and location:

(b) (4)

S.

Date of study initiation: November 30, 1987

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug, lot #, and % purity: polidocanol, lot #04454, purity not provided

Formulation/vehicle: polidocanol dissolved in DMSO

Methods:

Strains/species/cell line: fibroblasts derived from newborn Chinese lung (CHL)

Dose selection criteria:

Basis of dose selection: preliminary toxicity test incorporating the maximum feasible concentration (5000×10^{-3} µl/ml).

Range finding studies: CHL dosed with $9.8-5000 \times 10^{-3}$ µl/ml in DMSO and incubated for 48 hr in the presence or absence of S9. Cell density was determined post-treatment as an assessment of toxicity. Based on significant growth inhibition (>50%) at higher doses, the doses for the pivotal assay were set at 3.75, 7.5, and 15×10^{-3} µl/ml without S9, and 2.5, 5, and 10×10^{-3} µl/ml with S9.

Test agent stability: stable for at least 1 month at room temperature.

Metabolic activation system: S9 fraction obtained from livers of rats induced with phenobarbital and 5,6-benzoflavone.

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 2.5 µg/ml, without S9)

benzo [a] pyrene (B[a]P, 5 µg/ml, with S9)

Comments: Eagle's MEM with 10% inactivated calf serum was used as culture medium.

Exposure conditions:

Incubation and sampling times: see study design.

Study design: As noted above, cultures (n=2) were exposed to polidocanol (3.75, 7.5, and 15×10^{-3} $\mu\text{l/ml}$ without S9, and 2.5, 5, and 10×10^{-3} $\mu\text{l/ml}$ with S9) for 24 (with and without S9) or 48 hr (without S9 only). *NOTE: Cultures in the metabolic activation group also had concomitant cultures without S9 as controls for the metabolic activation method.* Cells were then rinsed, fixed, stained with 0.1% crystal violet, and examined under a microscope for chromosomal abnormalities.

Analysis:

No. of replicates: 2 plates/test material

Counting method: 100 metaphase chromosomes/plate were examined for numerical (ploidy and incidence) and/or structural (gaps, breaks, exchanges, and fragmentations) abnormalities.

Criteria for positive results: A negative result was concluded for an incidence of $< 5\%$ abnormal cells and a false positive was concluded for an incidence of $5 < x < 10\%$. A positive result was concluded when the incidence of abnormal cell was $\geq 10\%$.

Summary of individual study findings:

Study validity: As expected, negative control cultures had a mean incidence of chromosomal abnormalities $< 5\%$, and positive control cultures had an incidence $> 20\%$. Therefore, this appears to have been a valid assay.

Study outcome: In the absence of metabolic activation, DMSO-treated cultures were considered negative and MNNG-treated cultures were considered positive at both 24 and 48 hr incubations. Although not clearly dose-related (and graded as negative based on an incidence of $< 5\%$), an increase in the number of cells with structural aberrations relative to controls was noted in polidocanol-treated cultures at both 24 and 48 hr. In addition, a dose-related increase in the number of polyploid cells was also noted in polidocanol-treated cells at 24 and 48 hr, and was most pronounced at 48 hr (only considered positive at 48 hr).

In the presence of metabolic activation, DMSO-treated cultures were considered negative and B[a]P-treated cultures were considered positive at 24 hr. Polidocanol-treated cultures were also considered negative, although a slight increase in polyploid cells was noted ($< 5\%$ incidence).

Therefore, polidocanol appears to be positive for numerical chromosomal aberrations in the absence of metabolic activation.

3. Study title: Mutagenicity testing of polidocanol in Ames *Salmonella*/microsome plate test

Key findings: Under the conditions of this assay, polidocanol was negative in the Ames *Salmonella* test, and this appears to have been a valid assay. These results were repeated in a confirmatory assay.

Study no: 63318

Study type (if not reflected in title): Ames test.

Volume #, and page #: Volume 26 of 50, pp. 3867-3887.

Conducting laboratory and location:

(b) (4)

Date of study initiation: June 16, 1986

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug, lot #, and % purity: polidocanol, batch #04450, purity not provided

Formulation/vehicle: polidocanol dissolved in DMSO

Methods:

Strains/species/cell line: Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, and TA1538

Dose selection criteria:

Basis of dose selection: A preliminary range finding toxicity study.

Range finding studies: Cultures of tester strain TA 100 were exposed to 12 concentrations of polidocanol ranging from 0-0.39 µl/plate. The background was reduced significantly in concentrations ≥ 0.097 µl/plate.

Test agent stability: not provided

Metabolic activation system: S9 fraction from rat liver homogenate obtained from a commercial source (), batch #06249 of 21.8.85) was added at a concentration of 10% to a buffered solution (S9 mix).

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls:

strain	S9	chemical	solvent	µg/plate
TA98	-	2-nitrofluorene	DMSO	10
	+	2-aminoanthracene	DMSO	2.5
TA100	-	sodium azide	water	10
	+	2-aminoanthracene	DMSO	2.5
TA1535	-	sodium azide	water	10
	+	2-aminoanthracene	DMSO	2.5
TA1537	-	9-aminoacridine	ethanol	50
	+	2-aminoanthracene	DMSO	2.5
TA1538	-	2-nitrofluorene	DMSO	10
	+	2-aminoanthracene	DMSO	2.5

Exposure conditions:

Incubation and sampling times: see study design.

Doses used in definitive study: 0, 0.00016, 0.0008, 0.004, 0.02, 0.10 µl/plate (confirmatory assay: 0.00032, 0.00016, 0.008, 0.04, 0.20 µl/plate)

Study design: Tester strains (n=3 plates/test material) were combined with nutrient broth containing histidine and biotin, along with the test material, and mixed and poured onto a minimal agar plate. Plates were incubated for 48 hr and number of histidine revertants tabulated. A confirmatory assay of the same design was also performed.

Analysis:

No. of replicates: 3 plates/test material

Counting method: not specified.

Criteria for positive results:

1. The solvent control yielded a number of revertants within historical range
2. A dose-related increase ($>2x$ solvent control level) in the number of revertants with 3 concentrations in at least one strain.
3. The result(s) must be reproducible in a confirmatory assay.

Summary of individual study findings:

Study validity: see criteria for positive results. Results in solvent and positive controls were as anticipated in all strains.

Study outcome: No significant ($\geq 2x$) increase in the mean number of revertants relative to solvent controls was noted in any concentration in any tester strain with or without S9. Mean number of revertants in positive controls was $>2x$ the solvent control levels in all strains with and without S9. These results were reproduced in a confirmatory assay. Therefore, under the conditions of this assay, polidocanol was negative in the Ames *Salmonella* test, and this appears to have been a valid assay.

Genetic toxicology summary: Polidocanol was negative in bacterial reverse mutation assays in *Salmonella* and *E. coli*, and in a micronucleus assay conducted in mice. Polidocanol induced numerical chromosomal aberrations in cultured newborn Chinese hamster lung fibroblasts in the absence of metabolic activation.

Genetic toxicology conclusions: The available genetic toxicology data suggest that polidocanol is a weak clastogen, based upon an increase in numerical chromosomal aberrations in cultured CHO cells in the absence of metabolic activation. Polidocanol was negative in bacterial reverse mutation assays and in an in vivo assay for clastogenic activity (micronucleus assay) conducted in mice, suggesting that the compound is not a strong genetic toxicant.

Labeling recommendations: See labeling portion of recommendations and conclusion, below.

VI. CARCINOGENICITY:

Carcinogenicity summary: No carcinogenicity studies were submitted to this IND.

Carcinogenicity conclusions: The sponsor has previously stated that NTP has done carcinogenicity studies in mice and rats, but adequacy of these studies was questioned.

However, given the fact that this drug is unlikely to be used chronically, these studies are not necessary.

Labeling Recommendations: See labeling portion of recommendations and conclusion, below.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

1. Study title: Examination of the influence of polidocanol on the pregnant rat and the fetus by intravenous administration.

Key study findings: Polidocanol was not teratogenic in rats under the conditions of this study.

Study no.: 8927/94

Volume # and page #: Reference 49, Volume 24 of 50, pp. 2680-3117.

Conducting laboratory and location:

(b) (4)

Date of study initiation: December 21, 1994

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug, lot #, and % purity: polidocanol, batch #24359, purity not provided

Formulation/vehicle: Aethoxysklerol® 0.5%, 1 ml contains 5 mg polidocanol, diluted in a solution with ethanol, disodium hydrogen orthophosphate dihydrate, potassium dihydrogen orthophosphate and water for injection.

Methods:

Species/strain: rat/Sprague-Dawley Mol: SPRD

Doses employed: for the definitive study, control (0.9% NaCl solution), 2, 4, 10 mg Aethoxysklerol/kg/day in 20 ml/kg body weight, followed by 0.5 ml of 0.9% NaCl solution per animal.

Route of administration: i.v. into the tail vein

Study design: Doses were chosen based on a dose ranging study in non-pregnant females (3-100 mg/kg). At ≥ 18 mg/kg, severe local toxicity at the tail injection site (i.e. swollen, necrotic areas of the tail) was noted such that no injection was feasible beyond day 5.

Systemic toxicity dyspnea, reduced motility, staggering gait was noted, and lethality occurred at ≥ 30 mg/kg. For the pivotal study, after acclimatization, sexually mature male rats served as breeding partners. Pregnant dams dosed during gestational days 6-17. Day 0 was defined by the day on which sperm was noted in the vaginal smear.

Number/sex/group: 24/dams/group

Parameters and endpoints evaluated: Dams were observed twice daily for behavior, external appearance, and mortality, as well as local injection site reactions. Body weight was recorded daily, as was food consumption. On gestation day (gd) 20, dams were necropsied and ovaries and uteri were examined and weighed. Fetuses were removed and the following examinations performed: alive and dead fetus count; sex and viability of the fetuses; number and size of resorptions; determination of corpora lutea in the ovaries, implantations, and location of fetuses; gravid uterus weights; fetus weights;

external fetal examinations; 50% of fetuses used for skeletal examination and 50% used for soft tissue examination.

Results:

Mortality: none.

Clinical signs: Local effects at the site of injection included blue discolorations, swelling, inflammatory reactions and/or necrosis that were slight at 2 mg/kg, and more severe at 4 mg/kg with indurations at the tail tip of two animals. The reaction was severe enough on one (n=4) to two (n=4) dosing days to prevent dosing for that day(s) between gd 15-17. At 10 mg/kg, reactions were more severe. Drug administration was not possible in that group for 1-5 (n=1 for 1 day, n=5 for 2 days, n=14 for 3 days, n=3 for 4 days, n=1 for 5 days) administration days between gd 11-17 in all 24 animals (see table). Loss of part of the tail was seen in a dose-related manner in treated groups.

Observation	Group 1 Control		Group 2 2 mg/kg		Group 3 4 mg/kg		Group 4 10 mg/kg	
	N	D	N	D	N	D	N	D
Tail								
Blue discoloured, swelling and/or inflammatory reaction	0	-	13	2-7	22	2-9	24	7-12
Necrosis								
single foci Ø approx. 5 mm	0	-	1	1	9	2-4	20	1-7
Ø approx. 5 mm	0	-	0	0	1	1	2	4-7
approx. 35 x 5 mm	0	-	1	2	4	1-6	12	1-8
loss of part of the tail	0	-	2	-	5	-	17	-
Induration	0	-	0	-	2	1-2	6	1-6
No application possible (between gestation days 11-17)	0	-	0	-	8	1-2	24	1-5

N = Number of affected dams of 24 dams evaluated

D = Number of gestation days affected after the 1st administration (▲ gestation day 6)

At 4 mg/kg, vocalization during administration on gd 10-12 was observed in one dam. Two of the 10 mg/kg animals exhibited staggering gait within 5 minutes of the first administration and lasting for up to 5 minutes. Vocalizations were also noted in 11 high dose dams at administration on one administration day each (gd 9, 10, 13, or 15). Body weight and weight change: Toward the end of the study the mean body weight tended to be slightly (circa 5%) reduced from the control value in all treatment groups, with no difference between the high dose group and the low dose group. At 4 mg/kg, body weight changes were slightly but significantly lower than control for gd 12-15. Body weight in the high dose group was significantly lower than controls on gd 15-18. Body weight change was reduced in this group for gd 9-12 and gd 12-15 as compared to controls. In my opinion the effects on body weight and weight gain were not sufficiently substantial as to be deemed dose-limiting.

Food consumption: Food consumption was slightly but significantly decreased as compared to controls in the 4 mg/kg dose group on gd 14. At 10 mg/kg, food consumption was significantly lower than control on gd 13-14.

Toxicokinetics: not performed

In-life observations: Local injection site observations included those noted under clinical signs.

Terminal and necroscopic evaluations:

Dams: No drug-related systemic changes were observed. Dilation of the renal pelvis was found in one dam in each of the 4 and 10 mg/kg groups and in one control animal. Gravid uterine weight was unchanged in treated groups as compared to control. The pregnancy rate was 96-100% in all groups. No differences were reported in the number of corpora lutea, implantation sites, resorptions, live fetuses, or pre-implantation loss (*Reviewer's comment: There appears to be an increase in early resorptions that was not statistically significant*).

Offspring: No external malformations or variations were reported. One dead fetus was seen in the high dose group (none in any other group). The report states that an increased number of "runts" was observed (pups weighing less than 70% of the mean for that litter) in the high dose group (7, versus 1 in the control group), but in the absence of any effect on the mean pup weight, or other abnormalities, I consider this to be meaningless. Note also that 10 mg/kg/day was found to be a NOEL in rats for effects on pups in an additional teratology study that was conducted (see reprotox study No. 4, below, for details).

No skeletal malformations were seen. Skeletal variations were similar in treated and control groups. Skeletal retardations included retarded ossification, incomplete or missing ossification in the skull, sternebrae, vertebral bodies, and metacarpal/tarsal bones. The latter were found in all treated groups "without any substance-related difference of biological relevance."

No soft tissue malformations were noted. Variations were consistent with background data, although an increased incidence (both fetal and litter) of 4th cerebral ventricle dilation was seen in the high dose group.

Summary of individual study findings: The NOEL for both systemic maternal effects and for fetal effects appears to have been 10 mg/kg. A NOEL for local effects (at the injection site) was not observed under the conditions of this study. Polidocanol was not teratogenic in this study.

2. Study title: Examination of the influence of polidocanol on the pregnant rabbit and the fetus by intravenous administration.

Key study findings: The NOEL for both maternal and fetal effects was 2 mg/kg. Polidocanol was not found to be teratogenic in this study, but fetal viability and body weight were reduced at 4 mg/kg and 10 mg/kg. The latter effects were probably secondary to maternal toxicity, but I must conclude from these data that polidocanol is embryotoxic.

Study no.: 8926/94

Volume # and page #: Reference 53, Volume 25 of 50, pp. 3396-3697.

Conducting laboratory and location: (b) (4)

Date of study initiation: December 28, 1994

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug, lot #, and % purity: polidocanol, batch #24359, purity not provided

Formulation/vehicle: Aethoxysklerol® 0.5%, 1 ml contains 5 mg polidocanol, diluted in a solution with ethanol, disodium hydrogen orthophosphate dihydrate, potassium dihydrogen orthophosphate and water for injection.

Methods:

Species/strain: Himalayan rabbits

Doses employed: for definitive study, control (0.9% NaCl solution), 2, 4, 10 mg Aethoxysklerol/kg/day in 5 ml/kg body weight, followed by 1 ml of 0.9% NaCl solution per animal.

Route of administration: IV in ear vein or hindlimb (saphenous) vein, from days 6 - 20 of gestation.

Study design: Doses were chosen based on a dose ranging study in non-pregnant females (0.3-10 mg/kg). Doses in the dose-ranging study were administered from days 1-11 into the ear vein, and from days 12-17 into the saphenous vein. Slight local intolerance was noted in the 0.3 mg/kg group. At ≥ 1.0 mg/kg, swollen, edematous, and/or necrotic areas at the injection sites were noted, findings that increased in severity and onset with dose. At ≥ 3.0 mg/kg, systemic toxicity such as dyspnea and staggered gait were noted, and spasmodic respiration, tremor, ataxia, and tonic-clonic convulsions occurred at 10 mg/kg.

For the pivotal study, after acclimatization, sexually mature male rabbits served as breeding partners. Pregnant dams dosed during gestational days 6-20 into the ear vein or the saphenous vein (alternating days). Day 0 was defined by the day on which sperm was noted in the vaginal smear.

Number/sex/group: 12/sex/group (*Reviewer's comment: The number of dams per treatment group is below the 16-20 recommended by the ICH guidelines*)

Parameters and endpoints evaluated: Dams were observed twice daily for behavior, external appearance, and mortality, as well as local injection site reactions. Body weight was recorded daily, as was food consumption. On gestation day 29, dams were necropsied and ovaries and uteri were examined and weighed. Fetuses were removed and the following examinations performed: alive and dead fetus count; sex and viability of the fetuses; number and size of resorptions; determination of corpora lutea in the ovaries, implantations, and location of fetuses; gravid uterus weights; fetus weights; external fetal examinations; skeletal and soft tissue examination of each fetus.

Results:

Mortality: One dam in the 4 mg/kg/day group died on gd 12. Four dams in the 10 mg/kg/day group died between gd 12-15. An additional two dams in each of those groups were sacrificed prematurely after aborting.

Clinical signs: Local effects at the site of injection included blue discolorations, swelling, inflammatory reactions and/or necrosis around the injection site. These ranged from

slight at 2 mg/kg, at which one dam had edema in the ear, to more severe at 4 mg/kg, with indurations at the tip of the ear of 4 animals, and a necrotic focus in one dam. At 10 mg/kg, effects were even more pronounced in a dose-related manner, consisting of loss of the ear tip, dark discoloration, swelling and necrosis in the hindlimb (see table).

Observation	Group 1 Control		Group 2 2 mg/kg		Group 3 4 mg/kg		Group 4 10 mg/kg	
	N	D	N	D	N	D	N	D
EAR(S)								
Blue discoloured (uni- or bilateral)	0	-	12	15-21	11	4-20	8	2-12
Swelling (uni- or bilateral)	0	-	10	4-19	10	6-19	11	2-20
Necrosis (uni- or bilateral)	0	-	9	2-12	9	3-12	8	11-20
Oedema	0	-	1	11	1	3	0	-
Necrotic focus	0	-	0	-	1	1	0	-
Loss of ear tip	0	-	0	-	0	-	1	1
Induration of the ear tip	0	-	0	-	4	1-12	7	1-10
HIND EXTREMITY (around the injection site)								
Dark-red discoloured (uni- or bilateral)	0	-	2	3-5	12	6-23	12	5-16
Swelling (uni- or bilateral)	0	-	0	-	1	5	0	-
Necrosis	0	-	0	-	1	2	4	1-11

N = Number of affected dams of 12 dams evaluated

D = Number of gestation days affected after the 1st administration (▲ gestation day 6)

Two dams in the 4 mg/kg/day group aborted on gd 21 and 26, respectively. Two dams in the 10 mg/kg/day group aborted on gd 25 and 26, respectively. No clinical signs were noted in any of these four animals.

In the dams that died during the study, no clinical signs were seen, except for two of the high dose animals: one presented in a lateral position with reduced motility, the other exhibited tonic-clonic convulsions and lateral position.

At 10 mg/kg/day, dyspnea, tonic-clonic convulsions (or disposition to show convulsions), staggering gait, and abdominal or lateral position were seen in most animals, beginning 5-20 minutes after dose administration and lasting for up to one hour on one to all administration days. Two animals exhibited reduced motility; one also had a head tilt, and the other had a hemorrhagic anogenital region.

Body weight: At 4 mg/kg/day, body weight was slightly less than control by 6-9% from gd 23 on, but was not statistically significant. Body weight change was stagnant or negative for intervals from gd 15-27, and was significantly different from control for gd 18-21. At 10 mg/kg day, body weight was also less than control by 5-11% from gd 23 on, but was still not statistically significant. Body weight change was negative in the high dose group for intervals from gd 12-27.

Food consumption: Food consumption at 4 mg/kg was decreased by 7-39% relative to control from gd 8 onward and was statistically significant on gd 12, 16-22, and 24. At 10 mg/kg, food consumption was decreased by 13-55% relative to control from gd 8 onward and was statistically significant on gd 10-13 and 15-22.

Toxicokinetics: not performed

In-life observations: see clinical signs above.

Terminal and necroscopic evaluations:

Dams: No effect of drug was seen on pregnancy rate. Gravid uterus weight was decreased at 4 and 10 mg/kg and was statistically significant only at 10 mg/kg. In the 4 mg/kg group, of the 9 surviving dams, 4 had all resorptions. In the 10 mg/kg group, of the 6 surviving dams, 4 had all resorptions. In both the 4 and 10 mg/kg groups, there was a significant difference in the number of dams with viable fetuses.

At 2 mg/kg, no effect was seen on the number of corpora lutea, implantations, resorptions, live fetuses, or pre-implantation loss, although one dam had only three corpora lutea, three implantation sites, all of which were early resorptions. At 4 mg/kg and 10 mg/kg, there was a dose-related increase in resorptions; both early resorptions and total resorptions were significantly increased compared to controls. Post-implantation loss was significantly increased, and the number of live fetuses was decreased (see table).

Parameter		Group 1 Control	Group 2 2 mg/kg	Group 3 4 mg/kg	Group 4 10 mg/kg
Dams with viable fetuses	N	12	11	* 5	**2
Dams with all resorptions	N	0	1	**4	**4
Corpora lutea	total per dam	95 7.9	91 7.6	77 8.6	41 6.8
Implantation sites	total per dam	88 7.3	80 6.7	72 8.0	41 6.8
Resorptions	total per dam	10 0.8	9 0.8	**38 4.2	**31 5.2
Early resorptions	total per dam	10 0.8	6 0.5	**36 4.0	**27 4.5
Late resorptions	total per dam	0 0.0	3 0.3	2 0.2	4 0.7
Live fetuses	total per dam	78 6.5	70 6.4	**33 6.6	**10 5.0
Pre-implantation loss	mean %	7.6	13.3	6.5	0.0
Post-implantation loss	mean %	13.2	14.9	53.9	74.1

p ≤ 0.05

p ≤ 0.01

The statistical comparison was conducted by comparing the respective value with the corpora lutea or with the number of implantation sites.

Offspring: At 4 mg/kg, fetal weight was decreased by 23-32% compared to controls; fetal weight was significantly less than control for females. At 10 mg/kg, fetal weight was decreased by 15-17%. The apparent lesser effect is skewed by the lower number of live fetuses from which the mean was calculated.

Two fetuses from the same litter at 4 mg/kg exhibited nephroptosis or downward displacement of the kidney, in the absence of other internal findings. The finding was considered to be possibly dose-related, since renal effects were observed on pathological examination in three dams at this dose.

One skeletal malformation (encephalocele) was observed at 4 mg/kg (same as that noted below as an external malformation). Skeletal variations included a statistically significant increase in the fetal incidence of enlarged fontanelle at 4 mg/kg. However, that variation occurred in only one litter, and also occurred in one litter at 2 mg/kg; this was considered to be within the range of background incidence. It was

also considered to be consistent with reduced fetal weight and likely to be due to retarded ossification. There was also incidences of unossified tarsus in the 2 mg/kg group (2/11 litters) and the 4 mg/kg group (2/5 litters). Skeletal retardations were similar in incidence in treated and control groups, and were reported to be within the range of background incidence.

The malformations noted included two litters with two fetuses each at 4 mg/kg (three fetuses with omphalocele, one with encephalocele). The report states their incidence to be significantly different from control, but they were still considered to be spontaneous. The fetal incidence of total malformations at this dose was statistically different from control, but the litter incidence was not.

One dead fetus was noted in each of the 2 and 4 mg/kg groups. That incidence was consistent with background.

In the control group, one runt was born in one litter out of 12 live litters. At 2 mg/kg, five runts were noted in three litters out of 11 live litters. At 4 mg/kg, three runts were noted in two litters out of five live litters. At 10 mg/kg, no runts were born, but there were only 2 live litters. The report states that this is within the range of background.

Summary of individual study findings: The apparent NOEL for both maternal and fetal effects was 2 mg/kg. Polidocanol was not found to not cause skeletal or visceral abnormalities in this study, but induced both maternal and fetal toxicity at exposures above 2 mg/kg, including reduced mean weight and reduced fetal survival. I must conclude from these data that polidocanol is embryotoxic.

3. Study title: Reproductive and developmental toxicity studies of polidocanol. (Fertility and early embryogenesis study in rats).

Key study findings: Based on the absence of significant treatment-related findings during in-life and terminal/necropsic observations of parents and fetuses, it appears that the NOEL for effects on reproductive performance of the parents and abnormalities of the resulting fetuses is \geq 10 mg/kg polidocanol, when administered intermittently (see table, below, for dosing frequency).

Study no.: ??? The Appended data table is numbered R-508, but it is unclear if this represents the study number or not.

Volume # and page #: Reference #46 (report) & 47 (individual data) Volume 23

Conducting laboratory and location: (b) (4)

Date of study initiation: November 18, 1993

GLP compliance: yes (x) no ()

QA reports: yes () no (x)

Drug, lot #, and % purity: polidocanol 1%, lot #29548/900, purity not provided

Formulation/vehicle: Aethoxysklerol 3%/vehicle = 72 mg sodium dihydrogen phosphate dihydrate, 25.5 mg potassium dihydrogen phosphate, 1.198 g absolute EtOH, q.s. to 30 ml in distilled water for injection.

Methods:

Species/strain: Crj:CD(SD)SPF rats

Doses employed: 0, 2.5, 5, and 10 mg/kg/0.025, 0.05, 0.1 ml/100 g bw, 2 ml/min (immediately prior to use, Aethoxysklerol 3% was diluted to 1% in vehicle)

Route of administration: i.v. into the tail vein

Study design: Dosing was performed intermittently over a 9 week period to minimize the severity of local irritation at the injection site.

group	total # doses	interval
control males	17	every 3 days for 6 weeks, then 1/week during last 3 weeks
2.5 mg/kg males	17	every 3 days for 6 weeks, then 1/week during last 3 weeks
5 mg/kg males	13	every 4-5 days for 6 weeks, then 1/week during last 3 weeks
10 mg/kg males	10	1/week for 9 weeks (first dose = week 0)
control females	14	every 3 days during pre-mating, then 1/day during GD 0-7.
2.5 mg/kg females	14	every 3 days during pre-mating, then 1/day during GD 0-7.
5 mg/kg females	12	every 4 days during pre-mating, then 1/day during GD 0-7.
10 mg/kg females	7	1/week during pre-mating, then 1/day on GD 0, 5, 6, & 7

Number/sex/group: 24/sex/group

Parameters and endpoints evaluated: Observed for mortality and clinical signs of toxicity 3 times daily during the administration period and once daily during other periods. Body weights and food consumption were recorded twice weekly during the pre-mating period, and females were weighed every other day during GD 0-7. Evidence of mating was determined by the presence of a vaginal plug or sperm in the vaginal smear (designated as GD 0). The following indices were calculated: copulation index for males and females [(# copulated animals/# animals housed together) x 100]; insemination index [(# males impregnating partner/# males successfully copulated with partner) x 100]; and fertility index [(# pregnant females/#copulated females) x 100]. Necropsies were performed on copulated males (testes and epididymides weighed) and females (20 days post-copulation). For pregnant females, the numbers of corpora lutea, implantations, live fetuses and embryonic/fetal deaths were determined. Live fetuses were evaluated for external and internal (soft tissue and skeletal) malformations.

Results:

Mortality: none

Clinical signs: Reddish urine reported in all female groups, and in males \geq 5 mg/kg, and labored breathing in 10 mg/kg males. Each group had irritation at the injection site, progressing to from reddish to purplish coloration and necrosis to loss of tail as dose increased. Recovery was not seen at the end of the administration period.

Body weight: 10 mg/kg males had significantly reduced body weight changes during the second half of the pre-mating period. No significant differences were noted in females.

Food consumption: Decreased in 10 mg/kg males.

Toxicokinetics: not performed

For fertility studies:

In-life observations: The copulation index for all groups was 100%. 1/24 non-pregnant pairs were noted in the control and 2.5 mg/kg groups, yielded insemination and fertility indices of 95.8%, while the 5 mg/kg and 10 mg/kg groups had indices of 100%.

Terminal and necroscopic evaluations: No treatment-related findings, other than the aforementioned adverse effects on the tail. No effects on testes or epididymides weights

in males or the number of estrous or duration of estrous cycle in females. No significant differences were noted in the numbers of corpora lutea, implantation sites, or live fetuses. Although not reported, there does appear to be a dose-related increase in the number of resorbed embryos. No treatment-related differences were noted in the sex ratio of body weights of fetuses, nor in the number of animals with abnormalities or variations (or in the number of individual abnormalities or variations).

Summary of individual study findings: Rats dosed intermittently with polidocanol (up to 10 mg/kg i.v. into the tail vein) had dose-related increases in the incidence and severity of local irritant effects on the tail. Decreased body weight gain and food consumption in HD males was attributed to the severe adverse effects on the tail (necrosis/loss of tail). No treatment-related effects on reproductive performance were noted among treated and control groups. No treatment-related effects on the sex ratio, body weights, or external/internal abnormalities of fetuses were noted among treated and control groups. Based on the absence of significant treatment-related findings in these parameters, it appears that the NOEL for effects on reproductive performance and the resulting fetuses is ≥ 10 mg/kg polidocanol. Note: It should be noted that dosing did not occur daily in this study, as is typical for reproductive toxicology studies. However, given the intermittent nature of the proposed clinical use, these data may be summarized in the label of the product.

4. Study title: Reproductive and developmental toxicity studies of polidocanol. Note: This study report concerned teratology studies conducted in both rats and rabbits, and a perinatal development study in rats. For clarity, the portion dealing with teratology in rats was reviewed in this section, the portion dealing with teratology in rabbits was reviewed under point 5, below, and the portion dealing with perinatal development in rats was reviewed under point 6, below.

Key study findings: The primary adverse effects noted in dams dosed on GD 7-17 with polidocanol solution i.v. at up to 10 mg/kg was local effects on the tail that increased in severity with dose. Adverse effects on live-born pups were limited to body weight development reductions, which did not correspond to any other clinical sign of toxicity (i.e. viability, neurobehavioral pattern, reproductive performance). Therefore, under the conditions of this study, polidocanol at up to 10 mg/kg was not teratogenic in the rat.

Study no.: ??? The Appended data table is numbered R-509, but it is unclear if this represents the study number or not.

Volume # and page #: Reference #46 (report) & 48 (individual data) Volume 23

Conducting laboratory and location: (b) (4)

Date of study initiation: November 18, 1993

GLP compliance: yes (x) no ()

QA reports: yes () no (x)

Drug, lot #, and % purity: polidocanol 1%, lot #29548/900, purity not provided

Formulation/vehicle: Aethoxysklerol 3%/vehicle = 72 mg sodium dihydrogen phosphate dihydrate, 25.5 mg potassium dihydrogen phosphate, 1.198 g absolute EtOH, q.s. to 30 ml in distilled water for injection

Methods:

Species/strain: Crj:CD(SD)SPF rats

Doses employed: 0, 2.5, 5, and 10 mg/kg/day, 0.025, 0.05, 0.1 ml/100 g bw, 2 ml/min (immediately prior to use, Aethoxysklerol 3% was diluted to 1% in vehicle)

Route of administration: i.v. into the tail vein

Study design:

group	total # doses	interval
control	11	Once daily from GD 7-17
2.5 mg/kg	11	Once daily from GD 7-17
5 mg/kg	11	Once daily from GD 7-17
10 mg/kg group 1	6	Once daily from GD 7-12
10 mg/kg group 2	6	Once daily from GD 12-17

Number/sex/group: 36/females/group, except for 10 mg/kg which was broken into 2 groups of 27 females/group.

Parameters and endpoints evaluated: Observed for mortality and clinical signs of toxicity 3 times daily during the administration period and once daily during other periods. Body weights were recorded on GD 0, 4, 7-17, and 20, and twice weekly postpartum. Food consumption was recorded every 2-3 times/week. Dams and their fetuses delivered via cesarean section (n=24/dose group, except n=15 for HD groups) were observed just as copulated females in the fertility study above. In females in the spontaneous delivery group (n=12/dose group), parturition condition was observed and length of gestation and delivery index [(# females with live pups/# pregnant females) x 100] were determined. Dams were allowed to lactate pups for 21 days for evaluation of pup suckling condition, and then necropsied for recording the number of implantation sites. Live-birth [(# live born/#implantation sites) x 100], day 4 viability [(# pups alive on day 4/# live-born pups) x 100], weaning [(# live weanlings at day 21/# pups alive on day 4) x 100] and still-birth indices [(# stillborn pups/# of pups born) x 100] were determined. Each litter was randomly reduced to 8 pups (4/sex when feasible) for observation, and the remainder necropsied. Pups were examined for internal and/or external abnormalities. The remaining 8 pups/group were observed up to day 70 post-partum for behavioral and clinical assessments. Reproductive performance tests were performed on 1 rat/sex/group and copulation and fertility indices determined. Live F₂ fetuses from copulated F₁ females were examined for malformations and body weights recorded.

Results:

Mortality: none

Clinical signs: Reddish urine reported in all groups. Irritation at the tail injection site as previously described was noted in all polidocanol-treated groups. Similar to the fertility study in rats, local adverse effects at the tail (reddish to purplish color, necrosis, loss of tail) were noted in all polidocanol-treated groups, and the severity of these effects increased with dose.

Body weight: No significant differences were noted in mean body weights. Body weight gains were significantly reduced in 5 mg/kg and 10 mg/kg (latter-half administration group) during GD 7-17 and GD 17-20 respectively. During the lactation period, significantly reduced body weights were noted in 5 mg/kg and 10 mg/kg (first-half administration group) animals during post-partum day 7-21 and 4-21 respectively. Food consumption: 5 mg/kg and 10 mg/kg (first-half administration group) were significantly lower during GD 8-17.

Toxicokinetics: not performed

For embryofetal development studies:

In-life observations: See clinical signs above.

Terminal and necroscopic evaluations:

Dams: Necroscopic findings included the previously mentioned tail effects in all polidocanol groups, and splenic hypertrophy in 1/27 animals in the 10 mg/kg (first-half administration group). The gestation index was 100% in all groups, and no significant differences were noted between control and treated groups in the gestation period duration, the stillbirth index, or the number and index of live-born pups. Cesarean section findings revealed no significant differences between control and treated groups in the numbers of corpora lutea, implantation sites, or live-born pups, or in implantation or embryonic/fetal death indices.

Offspring: No significant differences in sex ratio or body weights of fetuses, nor were external abnormalities noted. No significant differences between control and treated groups in the ratio of animals with visceral abnormalities were noted. Skeletal abnormalities were noted in 1/177 2.5 mg/kg fetus (fused sternbrae), and 1/113 10 mg/kg (latter-half administration group, fusion of the cervical vertebral arch and thoracic vertebral arch, fusion of costal and fusion of thoracic vertebral arch). All other effects were noted among control and treated groups with no significant difference in incidence. With respect to live-born pups, no significant differences in body weights or sex ratio were noted, nor were any external malformations reported. Body weight development of these pups was decreased from postpartum day (PPD) 7-14 and 21-63 in 2.5 mg/kg males, and PPD 4-63 in 5 and 10 mg/kg males, and PPD 7-35 in females. No treatment-related effects were noted in viability or weaning indices, neurobehavioral patterns, or reproductive performance of developing pups.

Summary of individual study findings: The primary adverse effects noted in dams dosed GD 7-17 with polidocanol solution i.v. at up to 10 mg/kg was local effects on the tail that increased in severity with dose. No significant signs of systemic toxicity were noted among all groups. No significant differences were noted in the gestation period duration, the stillbirth index, or the number and index of live-born pups, or in the numbers of corpora lutea, implantation sites, or live-born pups, or in implantation or embryonic/fetal death indices. No significant external or internal effects were noted in live fetuses (cesarean) or pups (live-born). Body weight development was decreased postpartum in pups from polidocanol treated animals, but no effect on viability or weaning indices, behavioral patterns, or reproductive performance of developing pups were noted. Therefore, under the conditions of this study, polidocanol at up to 10 mg/kg was not teratogenic in the rat.

5. Study title: Reproductive and developmental toxicity studies of polidocanol. Note: This study report concerned teratology studies conducted in both rats and rabbits, and a perinatal development study in rats. For clarity, the portion dealing with teratology in rats was reviewed under point 4, above, the portion dealing with teratology in rabbits was reviewed under point 5,

here, and the portion dealing with perinatal development in rats was reviewed under point 6, below. Also, note that some of the individual-animal data appear to be missing from the submission (e.g., rabbit data). These data will be briefly reviewed, but the quality of the data, as submitted, should be regarded as being suspect.

Key study findings: Substantial fetal toxicity was observed in this study, including reduced fetal survival, but this was probably secondary to maternal toxicity. In a supporting study, in which dams were dosed over four day periods (with separate groups of animals dosed on gestation days 6-9, 9-12, 12-15, and 15-18), there were no significant differences between control and any treatment group in fetal survival, fetal weight, external malformations, or visceral or skeletal anomalies. In my opinion data from this study are of little value (see below for details). The rabbit teratology study conducted under point two of the reproductive toxicology section, above (study No. 8926/94), appears to have been a better study, and I will base my conclusions and labeling recommendation on that study.

Study no.: ??? The Appended data table is numbered R-509, but it is unclear if this represents the study number or not.

Volume # and page #: Reference #46 (report) & 48 (individual data) Volume 23

Conducting laboratory and location: (b) (4)

Date of study initiation: November 18, 1993

GLP compliance: yes (x) no ()

QA reports: yes () no (x)

Drug, lot #, and % purity: polidocanol 1%, lot #29548/900, purity not provided

Formulation/vehicle: Aethoxysklerol 3%/vehicle = 72 mg sodium dihydrogen phosphate dihydrate, 25.5 mg potassium dihydrogen phosphate, 1.198 g absolute EtOH, q.s. to 30 ml in distilled water for injection

Methods:

Species/strain: Kbl:NZW SPF rabbits

Doses/volume employed: 0, 1.25, 2.5, and 5 mg/kg/day, 1, 0.25, 0.5, and 1 ml/kg bw, 2 mL/min (immediately prior to use, Aethoxysklerol 3% was diluted to 0.5 % in vehicle)

Route of administration: i.v. into the marginal ear vein

Study design:

group	total # doses	interval
control	13	Once daily from GD 6-18
1.25 mg/kg	13	Once daily from GD 6-18
2.5 mg/kg*	13	Once daily from GD 6-18
5 mg/kg group 1*	13	Once daily from GD 6-18
5 mg/kg group 2*	4	Once daily from GD 6-9
5 mg/kg group 3*	4	Once daily from GD 9-12
5 mg/kg group 4*	4	Once daily from GD 12-15
5 mg/kg group 5*	4	Once daily from GD 15 to 18

*According to the report, "it became impossible midway to administer 3/16 animals in the 2.5 mg/kg group and 11/16 animals in the 5 mg/kg group". Details of exactly when dosing of these animal was stopped was not located in the report. Evidently, it was

decided to study the test material further at 5 mg/kg, utilizing animals dosed over four day periods; those groups are designated 5 mg/kg groups 2-5 in the above table.

Number/sex/group: apparently 15/females/group

Parameters and endpoints evaluated: Observed for mortality and clinical signs of toxicity 3 times daily during the administration period and twice daily during other periods.

Body weights were recorded on days 0, 3, 6-18, and every two days thereafter. Food consumption was recorded on days 3 and 6, and every two days thereafter. Dams were necropsied in on day 28 post-copulation. Numbers of corpora lutea, implantations, live and dead fetuses, and placental weight were determined. Live fetuses were weighed and examined for external malformations. Half were examined for visceral abnormalities and half for skeletal anomalies.

Results:

Mortality: One animal in each treatment group was sacrificed in extremis. One animal at 2.5 mg/kg and 3 animals at 5 mg/kg were found dead.

Clinical signs: Irritation at the injection site was noted in all polidocanol-treated groups, and included hardening, thrombotic swelling, discoloration, and ulceration. The severity of these effects increased with dose. In some animals the local effects were of sufficient magnitude as to preclude further dosing following 3-11 injections.

Body weight: Apparently somewhat reduced in all treatment groups, in proportion to dosage, but details not located in the study report.

Food consumption: Significantly reduced on day 18 at 2.5 mg/kg and from day 10-16 at 5 mg/kg.

Toxicokinetics: not performed

In-life observations: See clinical signs above.

Terminal and necroscopic evaluations:

Dams: Necroscopic findings included the previously mentioned local effects on the ears (injection sites). In animals at scheduled sacrifice, macroscopic findings included light yellow discoloration of the liver and "small spleen". In animals found dead or killed in extremis, observations included dark-red coloration of the lung and thymus, dark-red foci in the stomach, thrombus in the blood vessels in the cervical region, and yellow discoloration and yellow foci of the liver. Fetal death rate was increased in dose-dependent manner at 2.5 mg/kg and 5 mg/kg; this effect was statistically significant at 5 mg/kg only. The post-implantation losses were 6.5%, 20.4%, and 58.7% in the control, 2.5 mg/kg, and 5 mg/kg groups, respectively. The mean litter sizes were 7.7 ± 2.3 , 7.5 ± 2.0 , 6.7 ± 3.0 , and 3.3 ± 3.6 in the control, 1.25 mg/kg, 2.5 mg/kg, and 5 mg/kg groups, respectively. Cesarean section findings revealed no significant differences between control and treated groups in the numbers of corpora lutea or implantation sites (of course, since dosing didn't commence until day 6).

Offspring: Note: Due to reduced fetal survival, the number of live fetuses examined from dams dosed with 5 mg/kg was so low (only 26 total, 12 examined for visceral anomalies and 14 examined for skeletal anomalies) that statistical analysis of those data may have lacked sufficient power to permit meaningful evaluation of the data. Mean fetal body

weight tended to be reduced in all treatment groups, but was only statistically significant for female pups in 2.5 mg/kg group. There were no statistically significant effects on external malformations or visceral or skeletal anomalies, although there seemed to be trends toward increased incidences of each of these observations with increasing dosage. External malformations were observed in 0%, 1%, 5%, and 12% of fetuses in the control, 1.25, 2.5, and 5 mg/kg groups, respectively. The malformations observed included flexion contracture of the wrist joint, omphalocele, and oligodactyly of forelimbs combined with small hindlimbs. Visceral anomalies occurred in 12%, 10%, 6%, and 25% of fetuses in the control, 1.25, 2.5, and 5 mg/kg groups, respectively, and included thymic remnant in the neck, abnormal lobation of the lung, and aberrant right subclavian artery. Skeletal anomalies occurred in 0%, 0%, 5%, and 7% of fetuses in the control, 1.25, 2.5, and 5 mg/kg groups, respectively.

"Additional study": Due to excessive local effects at the injection site, particularly at 5 mg/kg, which prevented dosing over the entire 6-18 day period typically utilized in rabbit teratology studies, the sponsor conducted a follow-up study in which the animals were dosed with 5 mg/kg over 4 day periods, with separate groups of animals dosed on gestation days 6-9, 9-12, 12-15, and 15-18. In this study, effects at the injection site were similar to those observed previously, but were of lesser magnitude due to the shorter duration of dosing. There were no significant differences between control and any treatment group in fetal survival, fetal weight, external malformations, or visceral or skeletal anomalies.

Reviewer's comment: In my opinion data from this study are of little value due to the facts that the submission lacked "individual animal" data (the report was apparently translated from Japanese, and was sketchy and incomplete), due to intolerance of the animals to the intended dosage regimen, and due to presumably inadequate statistical power of data from the high-dose group (due to the small number of live pups in that group). Fetal survival was clearly reduced in the high-dose group, but this reflects maternal stress, and does not necessarily indicate a direct effect of the test material. The rabbit teratology study conducted under point two of the reproductive toxicology section, above (study No. 8926/94), appears to have been a better study, and I will base my conclusions and labeling recommendation on that study.

Summary of individual study findings: One animal (F0 female) in each treatment group was sacrificed in extremis, and one animal at 2.5 mg/kg and 3 animals at 5 mg/kg were found dead. Irritation at the injection site was noted in all polidocanol-treated groups in a dose-dependent manner, and included hardening, thrombotic swelling, discoloration, and ulceration. Necroscopic findings in dams included light yellow discoloration of the liver and "small spleen". In animals found dead or killed in extremis, observations included dark-red coloration of the lung and thymus, dark-red foci in the stomach, thrombus in the blood vessels in the cervical region, and yellow discoloration and yellow foci of the liver. Fetal death rate was increased in a dose-dependent manner at 2.5 mg/kg and 5 mg/kg; this effect was statistically significant at 5 mg/kg only. The post-implantation losses were 6.5%, 20.4%, and 58.7% in the control, 2.5 mg/kg, and 5 mg/kg groups, respectively. The mean litter sizes were 7.7 ± 2.3 , 7.5 ± 2.0 , 6.7 ± 3.0 , and 3.3 ± 3.6 in the control, 1.25 mg/kg, 2.5 mg/kg, and 5 mg/kg groups, respectively. Mean fetal body weight tended to be reduced in all treatment groups. There were no statistically significant effects on external malformations or visceral or skeletal anomalies, although there seemed to be trends toward increased incidences of each of these observations with increasing dosage.

External malformations were observed in 0%, 1%, 5%, and 12% of fetuses in the control, 1.25, 2.5, and 5 mg/kg groups, respectively. The malformations observed included flexion contracture of the wrist joint, omphalocele, and oligodactyly of forelimbs combined with small hindlimbs. Visceral anomalies occurred in 12%, 10%, 6%, and 25% of fetuses in the control, 1.25, 2.5, and 5 mg/kg groups, respectively, and included thymic remnant in the neck, abnormal lobation of the lung, and aberrant right subclavian artery. Skeletal anomalies occurred in 0%, 0%, 5%, and 7% of fetuses in the control, 1.25, 2.5, and 5 mg/kg groups, respectively. In a subsequent study, in which dams were dosed over four day periods (with separate groups of animals dosed on gestation days 6-9, 9-12, 12-15, and 15-18), there were no significant differences between control and any treatment group in fetal survival, fetal weight, external malformations, or visceral or skeletal anomalies.

6. Study title: Reproductive and developmental toxicity studies of polidocanol. Note: This study report concerned teratology studies conducted in both rats and rabbits, and a perinatal development study in rats. For clarity, the portion dealing with teratology in rats was reviewed under point 4, above, the portion dealing with teratology in rabbits was reviewed under point 5, above, and the portion dealing with perinatal development in rats was reviewed here.

Key study findings: With the exception of local effects at the injection site, polidocanol, administered i.v. at up to 10 mg/kg on an irregular schedule over the period from GD 17 to day 21 postpartum had no effects on the ability of pregnant rats to deliver or rear, or on gross external anatomy or reproductive function or F1 animals, or on gross external anatomy of F2 animals. Therefore, under the conditions of this study, polidocanol at up to 10 mg/kg did not induce adverse effects on perinatal development in the rat.

Study no.: ??? The Appended data table is numbered R-509, but it is unclear if this represents the study number or not.

Volume # and page #: Reference #46 (report) & 48 (individual data) Volume 23

Conducting laboratory and location: (b) (4)

Date of study initiation: November 18, 1993

GLP compliance: yes (x) no ()

QA reports: yes () no (x)

Drug, lot #, and % purity: polidocanol 1%, lot #29548/900, purity not provided

Formulation/vehicle: Aethoxysklerol 3%/vehicle = 72 mg sodium dihydrogen phosphate dihydrate, 25.5 mg potassium dihydrogen phosphate, 1.198 g absolute EtOH, q.s. to 30 ml in distilled water for injection

Methods:

Species/strain: Crj:CD(SD)SPF rats

Doses employed: 0, 2.5, 5, and 10 mg/kg/day, 0.025, 0.05, 0.1 ml/100 g bw, 2 ml/min (immediately prior to use, Aethoxysklerol 3% was diluted to 1% in vehicle)

Route of administration: i.v. into the tail vein

Study design:

group	total # doses	interval
control	14	Once every two days, from GD 17- PP* day 21

2.5 mg/kg	14	Once every two days, from GD 17- PP* day 21
5 mg/kg	9	Once every third or fourth day, from GD 17- PP* day 21
10 mg/kg	6	On days 17 and 21 of gestation and on days 1, 4, 7, and 14 PP

*PP indicates post-partum

Number/sex/group: 24/females/group

Parameters and endpoints evaluated: Observed for mortality and clinical signs of toxicity 3 times daily during the administration period and once daily during other periods. Body weights were recorded on GD 0, 4, 7, 11, 14, and daily from GD day 17-parturition. Food consumption was recorded every 2-3 times/week. Parturition condition was observed and length of gestation and delivery index [(# females with live pups/# pregnant females) x 100] were determined. Dams were allowed to lactate pups for 21 days for evaluation of pup suckling condition, and then necropsied for recording the number of implantation sites. Live-birth (# live born/#implantation sites) x 100], day 4 viability [(# pups alive on day 4/# live-born pups) x 100], weaning [(# live weanlings at day 21/# pups alive on day 4) x 100] and still-birth indices [(# stillborn pups/# of pups born) x 100] were determined. Each litter was randomly reduced to 8 pups (4/sex when feasible) for observation, and the pups were observed up to day 70 post-partum for behavioral and clinical assessments. Reproductive performance tests were performed on 1 rat/sex/group and copulation and fertility indices determined. Live F₂ fetuses from copulated F₁ females were examined for malformations and body weights recorded.

Results:

Mortality: No remarkable effects.

Clinical signs: Reddish urine reported in all groups; the incidence of this observation increased with increasing dosage. Irritation at the tail injection site as previously described was noted in all polidocanol-treated groups. Similar to the fertility study in rats, local adverse effects at the tail (reddish to purplish color, necrosis, loss of tail) were noted in all polidocanol-treated groups, and the severity of these effects increased with dose.

Body weight: Data not located in report.

Food consumption: 5 mg/kg and 10 mg/kg were significantly lower on day 17 PP.

Toxicokinetics: not performed

For embryofetal development studies:

In-life observations: See clinical signs above.

Terminal and necroscopic evaluations:

Dams (F0): Necroscopic findings included the previously mentioned tail effects in all polidocanol groups. The gestation index was 100% in all groups, and no significant differences were noted between control and treated groups in the gestation period duration, the stillbirth index, or the number and index of live-born pups. No effects on maternal behavior (pup retrieval, nest building, lactation behavior).

Offspring (F1): No significant differences in sex ratio or external malformations. Significantly reduced mean body weight in F1 female from HD group on day 70 PP (257.8 ± 21.2 g vs. 278.1 ± 17.7 g in HD and control F1 females, respectively). No effect on pup survival. No remarkable effects on external differentiation parameters (pinna detachment, incisor eruption, etc.). No treatment-related effects were noted in reflex function or behavior (righting reflex, pupillary reflex, grooming, water maze test, etc.). No effects on reproductive performance of F1 animals and no external abnormalities in F2 pups.

Summary of individual study findings: The primary adverse effects noted in dams dosed with polidocanol solution i.v. at up to 10 mg/kg was local effects on the tail that increased in severity with dose. No clear signs of systemic toxicity were noted among all groups. No significant differences were noted in the gestation period duration, the stillbirth index, the mean number and index of live-born pups, or embryonic/fetal death indices. In F1 animals, no remarkable effects on external differentiation parameters, reflex function, behavior, or reproductive performance, and no external abnormalities in F2 pups. Therefore, under the conditions of this study, polidocanol at up to 10 mg/kg did not induce adverse effects on perinatal development in the rat.

Reproductive and developmental toxicology summary: Developmental reproductive toxicity testing was performed in rats and rabbits, and fertility and perinatal development were studied in rats. In rats, no evidence of fetal toxicity was observed, and the NOEL for both systemic maternal effects and for fetal effects was 10 mg/kg. In rabbits, the NOEL for both maternal and fetal effects was 2 mg/kg. Polidocanol did not cause skeletal or visceral abnormalities in rabbits, but induced both maternal and fetal toxicity at exposures above 2 mg/kg, including reduced mean weight and reduced fetal survival. The fetal toxicity observed in rabbits was probably secondary to maternal toxicity, but it must be concluded that polidocanol induced an embryocidal effect in rabbits. Under the conditions studied, polidocanol did not affect reproductive performance (fertility) or ability of rats to deliver and rear pups (perinatal development). It should be noted that dosing did not occur daily in these studies, as is typical for reproductive toxicology studies. However, given the intermittent nature of the proposed clinical use, these data may be summarized in the label of the product. A NOEL for local effects (at the injection site) was not observed in any of these studies, as the test material is an irritant.

Reproductive and developmental toxicology conclusions: Polidocanol was not teratogenic in rats or rabbits, although reduced fetal survival was observed in rabbits in which the dams were dosed over gestation days 6-20. The embryocidal effect observed in rabbits was probably secondary to maternal toxicity. Polidocanol did not affect reproductive performance (fertility) or ability of rats to deliver and rear pups (perinatal development).

Labeling recommendations: See labeling portion of recommendations and conclusion, below.

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: Intramuscular irritation test of polidocanol in rabbits.

Key study findings: Polidocanol 1% was considered an intramuscular irritant following a single intramuscular injection.

Study no: ML-326B

Volume # and page #: Reference #44, Volume 23 of 50, pp. 2301-2331.

Conducting laboratory and location:

(b) (4)

Date of study initiation: November 30, 1987

GLP compliance: yes (x) no ()

QA reports: yes (x) no (), BUT UNSIGNED

Drug, lot #, and % purity: polidocanol 1%, lot #23189, purity not provided

Formulation/vehicle: injectable solution containing 1% polidocanol and 5% EtOH. Other components not specified.

Methods: 9 male Japanese white rabbits (3 months old, 3.2-3.7 kg) were divided into 3 test groups to receive single intramuscular injections into the left vastus lateralis of 1 ml Aethoxysklerol 1%, 0.425% and 1.7% acetic acid (positive controls). Each animal was injected into the right vastus lateralis with normal saline as a negative control.

Observations and times: Body weights were recorded pre-dosing and 5 days-post dosing. Local and systemic signs of toxicity were monitored daily. At termination (day 5) a gross necropsy was performed on each animal and injected muscle weights were recorded, muscle dimensions recorded, and histology performed.

Results: No significant body weights noted. Tumefaction and induration were noted in 3/3 Polidocanol-treated animals 1 day post-dosing. Induration (but no tumefaction) was noted in 2/3 and 3/3 0.425% and 1.7% acetic acid groups. These effects were resolved within 4 days in Polidocanol and 1.7% acetic acid groups, and within 2 days in the 0.425% acetic acid group. At necropsy, white-gray areas were noted in the Polidocanol treated muscles in 2/3 animals, as well as 1/3 in the acetic acid groups (1.7% > 0.425%). Muscle weights were not significantly different among treated and control groups. Histologically, muscle fiber necrosis was noted on 2/3 Polidocanol-treated animals, accompanied by inflammatory cell infiltrate and hemorrhage of the muscle. Similar effects were noted in acetic acid-treated animals, with incidence and severity greater in 1.7% than 0.425% acetic acid groups.

Summary of individual study findings: Given the similarity of effects at the injection site in Polidocanol and acetic acid-treated groups, Polidocanol 1.0% was considered a local irritant to rabbit muscle.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Polidocanol damages local endothelial cells through surfactant action, resulting in replacement of the damaged vessel with connective tissue. The drug substance is administered

intravenously, rapidly distributed (but does not cross the blood-brain barrier), extensively metabolized, cleared within 48-72 hours in rats and dogs, and eliminated primarily in the urine, but with a substantial fraction in the feces.

The primary adverse finding in acute toxicology studies (other than mortality) was irritation and necrosis in the vicinity of the injection site, which was undoubtedly related to the desired clinical actions of the material (destruction of endothelium). The local reaction was intensified, but similar, when the material was injected subcutaneously (instead of intravenously), since the drug substance was cleared from the site more slowly. Observations made in repeat-dose toxicology studies largely coincided with observations from acute studies, no doubt at least in part because the repeat-dose studies conducted with polidocanol involved intermittent dosing (usually once or twice per week), rather than daily dosing. This was deemed necessary due to the toxicity of polidocanol at exposures similar to the proposed clinical exposure. Intermittent dosing in repeat-dose studies is acceptable because the proposed clinical use would not involve repeated dosing on a daily basis.

In a repeat-dose toxicology study in which rats were injected with the drug product intravenously at intervals of one week for 13 weeks at dosages of 1, 3, or 9 mg/kg polidocanol (approximately 0.17, 0.5, and 1.5 times the proposed clinical dose, respectively), injection site damage (discoloration, necrosis, scarring, ulceration) was noted that increased in severity with duration. The high dose (9 mg/kg) group had to be discontinued after the sixth dose due to the severity of these lesions. The histological findings associated with this damage were attributed to the pharmacologic effect of polidocanol. Changes in RBC parameters (decreased red blood cell count, hemoglobin, and hematocrit) were attributed to hemolysis after injection of the test material. Increased white blood cell count and alterations in plasma proteins were thought to be due to an inflammatory response at the injection site. The NOEL for systemic toxicity was considered to be 1 mg/kg weekly for 14 doses (0.17 times the maximum human dose based on body surface area).

In a repeat-dose toxicology study in which dogs were injected with the drug product intravenously every other day for 13 weeks at dosages of 1, 3, or 9 mg/kg polidocanol (approximately 0.5, 1.6, and 4.9 times the proposed clinical dose, respectively), the NOEL for systemic toxicity was considered to be 3 mg/kg. A NOEL for local effects was not observed. Transient salivation and emesis were noted in males ≥ 3 mg/kg and all treated female groups immediately after dosing, but these symptoms were reversible and did not increase in severity during the study. Hematuria was noted in high dose animals, which was considered to be associated with treatment-related hemolysis. Reductions in hematological parameters noted in high dose animals were also likely to have been associated with hemolysis. Local toxicity such as perivenous fibrosis was greater in all treated groups as compared to controls. This local damage at the injection site was noted to persist in animals dosed at 3 mg/kg or more throughout the 28-day recovery period.

In a battery of genetic toxicology studies, polidocanol was negative in bacterial reverse mutation assays in *Salmonella* and *E. coli*, and in a micronucleus assay conducted in mice. Polidocanol induced numerical chromosomal aberrations in cultured newborn Chinese hamster lung fibroblasts in the absence of metabolic activation. These data suggest that polidocanol is a weak clastogen, but not a strong genetic toxicant.

Developmental reproductive toxicity testing was performed in rats and rabbits, and fertility and perinatal development were studied in rats. In rats, no evidence of fetal toxicity was observed, and the NOEL for both systemic maternal effects and for fetal effects was 10 mg/kg. In rabbits, the NOEL for both maternal and fetal effects was 2 mg/kg. Polidocanol did not cause skeletal or visceral abnormalities in rabbits, but induced both maternal and fetal toxicity at exposures above 2 mg/kg, including reduced mean weight and reduced fetal survival. The fetal toxicity observed in rabbits was probably secondary to maternal toxicity, but it must be concluded that polidocanol induced an embryocidal effect in rabbits. Under the conditions studied, polidocanol did not affect reproductive performance (fertility) or ability of rats to deliver and rear pups (perinatal development). It should be noted that dosing did not occur daily in these studies, as is typical for reproductive toxicology studies. However, given the intermittent nature of the proposed clinical use, these data may be summarized in the label of the product. A NOEL for local effects (at the injection site) was not observed in any of these studies, as the test material is an irritant.

Unresolved toxicology Issues (if any): NA

Recommendations: This NDA is approvable with respect to pharmacologic and toxicologic concerns. It is recommended that the labeling be revised as indicated below.

Labeling recommendations: The following changes in the draft labeling are recommended:

1. Clinical Pharmacology: The following paragraph should be deleted, as it does not pertain to clinical pharmacology:

[REDACTED]

2. Carcinogenesis, Mutagenesis, Impairment of Fertility: The text in this section should be stricken and replaced with:

"Long-term studies to evaluate carcinogenic potential have not been conducted with polidocanol. Polidocanol was negative in bacterial reverse mutation assays in *Salmonella* and *E. coli*, and in a micronucleus assay conducted in mice. Polidocanol induced numerical chromosomal aberrations in cultured newborn Chinese hamster lung fibroblasts in the absence of metabolic activation,

[REDACTED]

Polidocanol did not affect reproductive performance (fertility) [REDACTED] of rats [REDACTED] when administered intermittently at dosages up to 10 mg/kg (approximately [REDACTED] maximum dose [REDACTED] on the basis of body surface area).

3. Pregnancy. The text in this section should be stricken and replaced with:

[REDACTED] Pregnancy category C. Polidocanol has been shown to have an embryocidal effect in rabbits when given in doses approximately equal to the human dose [REDACTED] on the basis of body surface area). This effect may have been secondary to maternal toxicity. There are no adequate and well-controlled studies in pregnant women. [REDACTED]

4. [REDACTED]

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ____ No ____

X. APPENDIX/ATTACHMENTS:

Addendum to review: NA

Other relevant materials (Studies not reviewed, appended consults, etc.): NA

Any compliance issues: NA

cc: list:

NDA 21-201

HFD-540

HFD-540/DivDirector/Wilkin

HFD-540/Deputy DivDirector/Kukich

HFD-540/SupPharm/Brown

HFD-540/Pharm/See

HFD-540/MO/Vaughan; Carr

HFD-540/CMC/Hathaway

HFD-540/PMS/Carrington

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

/s/

Norman See
5/5/04 03:26:05 PM
PHARMACOLOGIST

Paul Brown
5/6/04 06:16:19 PM
PHARMACOLOGIST

NDA 21-201

**Division of Dermatologic and Dental
Drug Products (HFD-540)**

**Pharmacology/Toxicology Checklist for
NDA Filing Meeting (11/24/03)**

Date: November 20, 2003

Reviewer: David Allen, Ph.D.

NDA Number: 21-201

Sponsor: Chemische Fabrik Kreussler & Co., GmbH

Product Name: Aethoxysklerol

Drug Substance(s): polidocanol

Indication: treatment of varicose veins

Route of Administration: intravenous

Date CDER Received: October 8, 2003

User Fee Due Date (if filed):

Expected Date of Draft Review (if filed):

(1) Does the pharmacology/toxicology section of the NDA appear to be organized in a manner that would allow a substantive review to be completed?

Yes.

(2) Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner to enable a timely and substantive review?

Yes.

(3) Is the pharmacology/toxicology section of the NDA sufficiently legible to permit a substantive review to be completed?

Yes.

(4) Based upon a cursory review, does the presentation of data appear to be appropriate (consider tables, graphs, completeness of study reports, inclusion of individual animal data, appropriateness of data analysis, etc.)?

Yes.

(5) Are all necessary nonclinical studies completed and submitted in this NDA?

Yes. Several of the reference tabs for the reproductive toxicology studies (fertility and peri/post-natal development, study #'s 47, 48, 51, 52, & 56) contain only Appendices of individual animal data.

In response to our request, the Sponsor submitted a statement specifying that the data tables in question are associated with the final report under Reference #46.

(6) Please itemize the pivotal nonclinical studies included in the NDA and indicate any important nonclinical studies that were omitted.

Pivotal studies included:

- A. Single-dose rodent (mice, rats)**
- B. Single-dose non-rodent (rabbit, dog)**
- C. Multiple-dose: rodent (7-day rats), (13-week rats)**
- D. Multiple-dose: nonrodent (4-week dogs), (13-week dogs)**
- E. Biodistribution and elimination (rats, dogs)**
- F. Teratology in rodent (rats) and non-rodent (rabbit); fertility (rat); peri-/postnatal development (rat)**
- G. Genetic toxicology studies (Ames test, chromosomal aberration assay, mouse bone marrow micronucleus assay).**

Pivotal studies omitted: None appear to be omitted. However, see #5 for confusion related to fertility and peri/post-natal development studies.

(7) Based upon a cursory review, do the pivotal nonclinical studies appear to have been adequately designed (e.g., appropriate numbers of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?

Yes.

(8) As appropriate, were the test materials utilized in the pivotal nonclinical studies identical to the drug product or drug substance proposed for commercial use (including impurity profiles)? If not, or if this matter is unclear, please comment.

No; different manufacturers were used to produce the drug substance and product in some of the nonclinical and pivotal clinical studies. However, the sponsor has performed a 7-day bridging study to compare the toxicities of polidocanol (drug substance) produced at the 2 manufacturing sites. In addition, the specifications provided for the drug substance from each manufacturer indicate that the substance used in the to-be-marketed formulation is of the highest purity, and thus would not be expected to contain any impurities not qualified from studies with the previous manufacturer's polidocanol.

However, according to the Review Chemist, possible degradation products in

Aethoxysklerol (polidocanol) are (b) (4)

The proposed product specification does not analyze for these degradation products, and thus there are no acceptance criteria for them. They are also excluded from the drug substance specification as being routinely below LOQ. However, the long-term stability studies were pretty consistent in finding (b) (4) **ppm in the d.p., and there were spikes up to above** (b) (4) **ppm. One lot showed a value for** (b) (4) **of over** (b) (4) **ppm.**

Therefore, the sponsor should propose appropriate limits and supply data and reasoning for supporting the proposed limits. According to OSHA, the amount of (b) (4) **to**

which workers can be exposed over an 8-hour work day is 0.75 ppm. However, it should be noted that these exposures are related to workplace exposures and not likely intended for i.v. exposure scenarios.

In response to our request, the Sponsor has provided a review of the toxicities associated with the potential impurities/degradation products, and the levels of each anticipated in Aethoxysklerol.

(9) Based upon a cursory review, do the excipients appear to have been adequately qualified?
Yes.

(10) Was the route of administration used in the nonclinical studies the same as the intended clinical route of administration?
Yes.

(11) Has proposed draft labeling been submitted?
Yes.

(12) From a pharmacology/toxicology perspective, should this NDA be filed? If not, or if you have additional concerns, please indicate your recommendations in the form of draft comments that may be transmitted to the sponsor.

This NDA will be considered fileable if the following information is received before the filing date:

- 1. Please propose appropriate limits for all degradation products and supply data and reasoning for supporting the proposed limits.**

Sponsor's response: A review of the toxicities associated with the potential impurities/degradation products, and the levels of each anticipated in Aethoxysklerol were provided. Specification limits will be provided under separate cover in response to CMC inquiry.

- 2. The tabular data that comprises references 47, 48, 51, 52, & 56 should be linked to their associated GLP study reports. It appears that these tables may be associated with a study report included as reference #46. If these tables are not associated with reference #46, then the study reports associated with these tables should be submitted to the NDA.**

Sponsor's response: The tabular data in question is to be associated with reference #46.

Reviewing Pharmacologist

Date Signed

Pharmacology Supervisor

Date Signed

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

/s/

David G. Allen
11/25/03 09:01:57 AM
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