CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20148

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original NDA Review

John J. Jessop, Ph.D., Pharmacologist

NDA#: 20-148

Review Date: January 29, 1997

Date of Submission: May 20, 1996

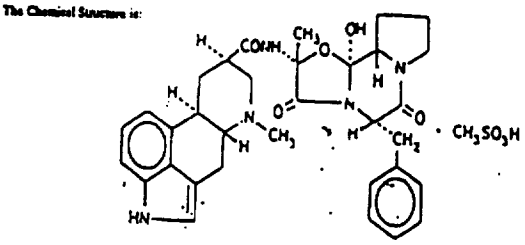
Due Date: May 20, 1997 (NDA was designated as "standard")

Sponsor: Sandoz Pharmaceuticals Corporation
59 Route 10
East Hanover, New Jersey 07936-1080

Drug: D.H.E.-45® (Dihydroergotamine Mesylate, (USP) Nasal Spray

Structure:

(Dihydroergotamine Mesylate, (USP) Nasal Spray)



Chemical Name: Ergotamine-3', 6', 18-trione, 9, 10-dihydro-12'-hydroxy-2'-methyl-5'-(phenyl-methyl)-,(5'∝)-monomethanesulfonate.

Molecular Formula: $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$

Molecular Weight: 679.79

Pharmacological Category: Synthetic Ergot Alkaloid

Indication: Symptomatic treatment of migraine headaches in adults

Related INDs/NDAs:

Proposed Clinical Use:

The proposed clinical use of DHE-45® is as follows: Initially, the proposed dose is one puff from intranasal delivery system to each nostril (0.5 mg DHE and 1.25 mg caffeine per puff, total dose 1 mg DHE for 2 nostrils). This dose can be repeated, if necessary, in 15 minutes. The proposed maximum total dose per headache is 2 mg DHE and 5 mg caffeine. The proposed maximum total dose per 24 hour period is 4 mg DHE and 10 mg caffeine. The proposed maximum dose per week is 12 mg DHE and 30 mg caffeine.

History of NDA 20-148

Original submission

The original NDA 20-148 was filed with the Division in May of 1991 and review completed in March of 1995. I inherited the NDA from Dr. Andrea Powell at the end of 1994.

This NDA proposed the administration of DHE-45® by the intranasal route for the treatment of migraine. The drug had previously been approved by the F.D.A. for treatment of migraine by the intravenous (i.v.) and intramuscular (i.m.) routes only. However, administration by the intranasal route required a separate NDA and introduced unique safety concerns associated with the nasal cavity that were not relevant to the i.v. and s.c. routes of administration. Administration of the drug by the intranasal route introduced a different drug exposure pattern from that of i.v. or i.m. administration that increased the Division's level of concern for risk of toxicity to the nasal cavity. Additionally it was felt that availability of an intranasal form of DHE-45®, a method of administration allowing fairly easy self-administration, would most likely dramatically increase the number of patients that would be exposed to the drug once approval was granted. Therefore the Division concluded that, although the drug had been on the market by the i.v. and i.m. route of administration, the introduction of the drug by the intrasal route of administration mandated that certain toxicology studies must be completed that were specifically designed to evaluate the safety of DHE-45® in terms of toxicity to the nasal cavity.

Upon completion of this review, I recommended that the NDA be regarded as "not approvable" due to a number of problems and toxicological effects outlined below in the "previous review and conclusions" section.

Redacted 1

page(s) of trade secret and/or confidential commercial information

When the sponsor discovered that the Division intended to classify NDA 20-148 as "not approvable", they chose to withdraw the NDA, with the intention of re-submitting at a later time.

Period from NDA withdrawal by the sponsor to resubmission of the NDA
Supplement to NDA 20-148; received October 24, 1995

In October of 1995, the Division received a supplement to NDA 20-148 in which the sponsor requested a meeting to discuss resubmission of the NDA. This submission also included new mutagenicity data, a report generated by a Pathology Working Group (PWG) put together by the sponsor to re-review the nasal cavity histopathology data from the mouse, rat and Cynomolgus monkey studies submitted with the original NDA and a number of scientific reprints on the subject of nasal cavity pathology in animal studies. According to the sponsor, the purpose of the PWG was to "further evaluate the treatment-related findings reported by the study pathologists and to determine the potential for these changes to progress to neoplasia." The PWG was chaired by The submission also included a report of adverse drug reactions from clinical studies in which DHE-45® Nasal Spray and other drugs containing similar ingredients have been administered to patients, with special emphasis on carcinogenicity issues. Finally, the submission included a request that the sponsor be allowed to complete only In this submission the sponsor stated that these

new mutagenicity data, the PWG report and other included information should support their hypothesis that "...the nasal lesions observed will not progress or transform to neoplasias..."

To summarize the findings of the PWG, the pathologists agreed with the original pathology report of the 1-month intranasal mouse study, determined that one of the high dose male animals in the 3-month mouse study did not present with focal squamous cell metaplasia, agreed with the original pathologist's report for the 1-month rat study, found three additional animals in the 3-month rat study that they diagnosed with focal squamous metaplasias that were missed by the original study pathologist, and determined that the lesion found in the high dose monkey was probably not a squamous metaplasia. The most significant finding reported by the PWG was their detailed description of the focal squamous cell metaplasias. With respect to characterization of these lesions, the PWG stated that "Although inflammation and squamous metaplasia may contribute to chemically-induced carcinogenesis, more frequently they represent an adaptive response in the upper respiratory tract of rodents in toxicology studies." They went on to state "Epithelial hyperplasia considered to represent a precursor to nasal tumors is often nodular, polyploid or papillary, while squamous metaplastic changes of concern are accompanied by extensive keratinization (Brown, et al., 1991). Cells within the foci of hyperplasia/metaplasia may demonstrate varying degrees of altered differentiation, cellular atypia, mitotic alteration and dysplasia (Boorman and Morgan, 1990). None of these features was observed in the present studies with subchronic exposure to DHE-45® Nasal Spray in mice, rats and monkeys."

In the original NDA submission, there was no detailed characterization of the hyperplasias or metaplasias included. However, in this PWG report the sponsor included information for the first time indicating that the lesions caused by intranasal administration of DHE-45® were not of the type (altered differentiation, cellular atypia, mitotic alteration and dysplasia) normally associated with a pre-neoplastic condition.

In this supplement, the sponsor also proposed to carry out the following additional toxicology studies in the rat:

In this Supplement the sponsor requested that the requirement for a study be waived because: (" " is a quote from the sponsor)

This Supplement also contained additional genetic toxicology study results, including results of newly completed bacterial gene mutation assay (Ames test), a human lymphocyte assay and a mouse micronucleus test. The submision of these data completed the required battery of genetic toxicology studies.

I wrote a review of this submission (see Attachment #3; attached review completed October 31, 1995) in which I 1) recommended that Aisar Atrakchi, Ph.D., the Division expert in genetic toxicology, review the genetox data submitted in this Supplement (Dr. Atrakchi's review is addressed in a separate section of this review entitled "Genetic Toxicology Update"), 2) recommended that Dr. Ron Moch, Veterinary Pathologist, CFSAN, be requested to review the pathology data and report of the PWG (Dr. Moch's review is addressed in the section "Nasal pathology results asssociated with interim sacrifice of rat CAR study animals" included in this review), 3) questioned the validity of the proposed 26-week intranasal toxicology study in rats in terms of the proposed number of rats/sex/group (3) and the proposed endpoint for determination of nasal cavity toxicology (the sponsor proposed examining the degree of proliferation of nasal mucosa epithelial cells rather than looking for squamous metaplasias) and 4) agreed with the sponsor that the systemic exposure to drug in the rat carcinogenicity study at the 0.8 mg/day dose (AUC 9-fold greater than human exposure at maximum proposed human dose) was probably adequate.

At this point, Dr. Atrakchi was asked to review the newly submitted genetic toxicology data, and Dr. Moch was asked to review the PWG report. Both were asked to submit written reviews and to attend the upcoming meeting with the sponsor.

Meeting on November 20, 1995 with sponsor to discuss NDA refiling issues and other issues included in NDA 20-148 Supplement of October 24, 1995

On November 20, 1995, a meeting was held with Sandoz to discuss refiling of NDA 20-148 as well as the other issues mentioned in the NDA 20-148 Supplement received October 24, 1995. That meeting was attended by representatives of the Division (Drs. Leber, Katz, Fitzgerald, Atrakchi and Jessop), Dr. Ron Moch, Chief, Pathology Branch, CFSAN, and various representatives from Sandoz including Ms. Susan Witham, Regulatory Affairs and Dr. William Iverson, Pathologist. Sandoz first discussed the findings reported in the report of the PWG. Their main points regarding this document were 1) that a number of the squamous metaplasia originally diagnosed by the study pathologist were found by the expert panel of the PWG to be incorrectly identified and 2) that the squamous metaplasia that were correctly identified in the original rat, mouse and monkey studies were not dysplasias, lesions with atypical cytological alterations that apparently constitute the pre-neoplastic stage of pathological development.

Then the sponsor asked the Division to outline requirements for refiling of NDA 20-148. After some discussion, it was decided that the sponsor would implement an

The Division questioned the value of this particular endpoint in light of the fact that the main concern up until that point in time was the occurrence of squamous metaplasias. After some

discussion, it was decided that the main concern was, in fact, the squamous metaplasias, and that this issue should be resolved satisfactorily with the 52-week interim sacrifice and histopathological evaluation of the nasal cavities of the animals from the ongoing rat carcinogenicity study.

The Division told the sponsor at this meeting that an answer regarding their request to waive

Addressing the sponsor's request to waive

Letter from the Division to Sandoz Pharmaceuticals Corporation: December 14, 1995

On December 14, 1995, a letter was sent from the Division to Sandoz, in which the sponsor was notified that the CAC Executive Committee had unanimously agreed that was acceptable for the ongoing study. The letter also requested that the sponsor notify the Division if mortality in the study increased and it appeared that there would be less than 20 rats/sex/group at study termination, so that a decision could be made as to whether or not early sacrifice of some groups should be implemented.

The sponsor was also notified in this letter that there would be a requirement for

Telephone conversation (January 4, 1996) between Dr. Ron Moch, Chief, Pathology Branch, CFSAN (pathology consultant for HFD-120 for NDA 20-148) and Dr. William Iverson, Pathologist, Sandoz

On January 4, 1996, Dr. Iverson telephoned Dr. Moch to inform him that, under the amended protocol for the

E-mail from Dr. Moch to Dr. Jessop, to inform him of telephone conversation with Dr. William Iversons: January 5, 1996 (see Attachment #7: attached copy of e-mail dated January 5, 1996)

On January 5, 1996, Dr. Ron Moch sent an e-mail to me, informing me that he had received a telephone call from Dr. William Iverson, Pathologist from Sandoz. In that conversation Dr. Iverson notified Dr. Moch that Sandoz had recently completed the

Redacted 1

pages of trade secret and/or confidential commercial information

New plasma protein binding data

New plasma protein binding data submitted in an NDA Supplement (received December 23, 1996) demonstrated that plasma protein binding of drug in mouse plasma (*in vitro*) was 95% (5% unbound drug) and in human plasma was about 91% (9% unbound). These data were for plasma drug levels ranging from 10-500 ng/ml, and apparently were independent of plasma-drug concentration.

Conclusions

NDA 20-148 proposed administration of DHE-45® by the intranasal route for treatment of migraine. The drug had already been approved for administration by the i.v. and i.m. route of administration for treatment of migraine. However, the Division concluded that administration by the intranasal route raised an additional concern unique to this route, that of potential nasal cavity toxicity. Also, it was felt that the availability of the drug by a route that provided such an easy and relatively painless method of administration would most likely result in a dramatic increase in the number of patients being exposed to DHE-45®. Therefore, considerable importance was placed on the results of the animal toxicology studies by the intranasal route of administration.

The original NDA 20-148 was determined to be "not approvable" in March of 1995. This decision was based on the following combination of problems: 1) an

DHE-45® Nasal Spray. When the sponsor found out that we had determined the NDA to be "not approvable" at that time, they withdrew the NDA.

Since that time, through a series of meetings and communications with the sponsor, it was agreed that the main safety issue hinged upon the formation of squamous metaplasia in the nasal cavities of the study animals. The sponsor has presented evidence, reviewed by the Division's consulting pathologist Dr. Ron Moch, that these squamous metaplasia were not of the type (keratinization, dysplasia) known to be pre-neoplastic in nature. Furthermore, the sponsor has completed a 52-week interim sacrifice of animals from their ongoing 2-year rat carcinogenicity study, and has reported that histopathological evaluation of the nasal cavities of these animals revealed that there were no squamous metaplasias present at this time point. This result indicates that these lesions do not persist chronically, which is consistent with their characterization by the sponsor as "adaptive" rather than "pre-neoplastic" in nature. Other inflammatory lesions, such as eosinophilic inclusions, have been evaluated by both Dr. Moch and Dr. William Iverson, pathologist from Sandoz, and they have concluded that these other lesions are consistent with a response to an irritant and do not indicate any serious risk to patients. Evaluation of plasma level data from these carcinogenicity study animals revealed that they had received an adequate local and systemic exposure to drug when compared to humans receiving the maximum recommended daily dose. Based on these findings, it is my conclusion that the sponsor has satisfactorily responded to the issue of the squamous metaplasia.

The sponsor has also completed the required genetic toxicology test battery and the required reproductive toxicology studies (they submitted fertility/reproductive performance and prenatal and postnatal studies with the refiled NDA). Genetic toxicology results indicate that the drug is probably a weak clastogen (See Attachment #12; Dr. Atrakchi's review). Reproductive toxicology testing is complete, and results can be found in the attached review by Dr. Edward Fisher (Attachment #15).

Finally, with respect to the studies, the sponsor has recently

Recommendations

With respect to approval
I recommend that NDA 20-148 be approved at this time.

Other recommendations

John J. Jessop 1-30-97

John J. Jessop, Ph.D., M.P.H.,
Pharmacologist

CC
NDA 20-148 (000)
Div file
HFD-120
/G.Fitzgerald/J.J.Jessop/R.Nighswander

I agree with the above recommendation

Gloria G. Fitzgerald
Team Leader
2/20/97

REVIEW AND EVALUATION OF TOXICOLOGY AND REPRODUCTION DATA

Kishena C. Wadhwani, Ph.D.

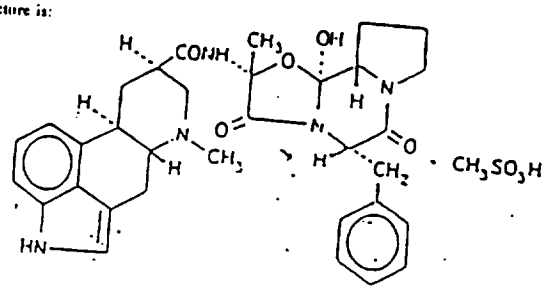
Date: February 17, 1995

NDA # 20,148

Drug: D.H.E. 45 (Dihydroergotamine Mesylate, USP) Nasal Spray

D.H.E. 45® is ergotamine hydrogenated in the 9, 10 position as the mesylate salt. D.H.E. 45® is known chemically as Ergotaman-3',6',18-trione,9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-,(5'α)-,monomethanesulfonate. Its molecular weight is 679.79 and its empirical formula is $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$).[1a]

The Chemical Structure is:



D.H.E. 45® Nasal Spray is provided for intra-nasal administration as a clear, colorless to faintly yellow solution in an amber glass ampul containing:[1b]

dihydroergotamine mesylate, USP	4.0 mg
caffeine, anhydrous USP	10.0 mg
dextrose, anhydrous USP	50.0 mg
carbon dioxide	qs
water for injection USP qs	1.0 mL

Pharmacologic Category: α adrenergic blocking agent, cranial vasoconstrictor, 5-HT_{1D} receptor agonist

Indication: Migraine Headache

Proposed clinical use: One puff of nasal spray from the delivery system per nostril. Each puff contains 0.5 mg DHE-45 and 1.25 mg caffeine. The proposed maximum total dose per 24 hour period is 4 mg DHE-45 and 10 mg caffeine.

Sponsor: SANDOZ Pharmaceuticals Corporation
East Hanover,
New Jersey 07936
Basle Switzerland Laboratories

Volumes Reviewed: 1.5 - 1.6

Sponsor's submission Date: January 17, 1992

Previously Reviewed Studies of this compound:

Original Review: NDA's #20,148 and #5,929: Biopharmakokinetics and Statistics.

Related IND's/NDA's:

Studies Currently Reviewed:

Toxicology:

Title: DHE-45 Acute toxicity study in mice, rats and rabbits (#201-007)
Volume: 1.5 (05-00581 - 05-00627)
Report Date: 10/19/1971
Drug Batch No. 8612
Sponsor's laboratory: **Sandoz LTD.**
CH-4002 Basle/Switzerland.

Title Dihydroergotamine mesylate (DHE-45)
A 26-week oral toxicity study in rats (#201-001)
Volume 1.5 (05-00628 - 05-00675)
Report Date 12/30/1969
Drug Batch No. 8612
Sponsor's laboratory: **Sandoz LTD.**
CH-4002 Basle/Switzerland.

Title Dihydroergotamine mesylate (DHE-45)
A 26-week oral toxicity study in dogs
Volume 1.5 (05-00676 - 05-00844)
Report Date 12/31/1969
Drug Batch No. 8612
Sponsor's laboratory: **Sandoz LTD.**
CH-4002 Basle/Switzerland.

Reproduction Study:

Title Dihydroergotamine methanesulphonate (DHE-45)
Fertility study in female rats (#201-008)

Volume 1.6 (05-00846 - 05-00967)
Report Date 11/11/1974
Drug Batch No. 72007
Sponsor's laboratory: **Sandoz LTD.**
CH-4002 Basle/Switzerland.

Title Dihydroergotamine methanesulphonate (DHE-45)
Fertility study in male rats (#201-009)
Volume 1.6 (05-00968 - 05-01159)
Report Date 03/11/1974
Drug Batch No. 72007
Sponsor's laboratory: **Sandoz LTD.**
CH-4002 Basle/Switzerland.

Title Dihydroergotamine Methanesulfonate (DHE-45)
Perinatal and postnatal study in rats (#201-003)
Volume 1.6 (05-01160 - 05-01195)
Report Date 06/19/1970
Drug Batch No. 8612
Sponsor's laboratory: **Sandoz LTD.**
CH-4002 Basle/Switzerland.

Title Dihydroergotamine Methanesulfonate (DHE-45)
Perinatal and postnatal study in rabbits (#201-004)
Volume 1.6 (05-01196 - 05-01234)
Report Date 12/21/1973 (Translated version from German document)
Drug Batch No. 8612
Sponsor's laboratory: **Sandoz LTD.**
CH-4002 Basle/Switzerland.

Title DHE-45
A teratology study in rats (#201-005)
Volume 1.6 (05-01235 - 05-01281)
Report Date 09/13/1971
Drug Batch No. 8612
Sponsor's laboratory: **Sandoz LTD.**
CH-4002 Basle/Switzerland.

Title	DHE-45
	A teratology study in rabbits (#201-006)
Volume	1.6 (05-01282 - 05-01342)
Report Date	09/13/1971
Drug Batch No.	8612
Sponsor's laboratory:	**Sandoz LTD.**
	CH-4002 Basle/Switzerland.

Title	DHE-45
	A teratology study in Stumptailed Macaques (#201-012)
Volume	1.6 (05-01343 - 05-01353)
Report Date	08/05/1976
Drug Batch No.	72006
Sponsor's laboratory:	**Sandoz LTD.**
	CH-4002 Basle/Switzerland.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

SUMMARY OF THE STUDY

The sponsor performed these studies without GLP. In addition, following discrepancies were noted: 1) administered routes used in these studies were oral, i.p, i.v. and s.c., although the proposed route was intra-nasal, and 2) caffeine, an excipient used in the proposed study, was not included in any of the toxicology and reproduction studies.

TOXICOLOGY

I. DHE-45 Acute toxicity study in mice, rats and rabbits (#201-007)

A. MATERIALS AND METHODS:

1) Chemicals: DHE-45,9,10-Dihydroergotamine methanesulfonate, batch # 8612, was prepared in different stock solutions as follows.

a. For intravenous route:

* *Mice*: **1%** DHE-45 solution (w/v; + inactive ingredients: 0.5% tartaric acid, 6% ethanol + ~5% glucose solution), pH = 3.5 (kept at 60 to 80°C before use.)

* *Rats:* **2%** DHE-45 solution (+ 1% tartaric acid, 6% ethanol + 5% glucose solution), pH = 3.5 (kept at 60 to 80°C before use.)

* *Rabbits:* **1.6%** DHE-45 solution (+ 18% ethanol, 4.4 % glucose solution), pH=4.5-5.0 (kept at 60 to 80°C before use)

b. For subcutaneous route:

* *Mice:* **5%** DHE-45 solution (+ 37% ethanol), pH = 4.5 (kept at 60 to 80oC before use.)

* *Rats:* **5%** DHE-45 solution (+37% ethanol), pH = 4.5 (kept at 60 to 80oC before use.)

* *Rabbits*: **1.5%** DHE-45 solution (+ 18% ethanol), pH=4.5 to 5.0 (kept at 60 to 80oC before use.)

c. For intraperitoneal route:

* *Mice* **1%** DHE-45 solution (~10% ethanol), pH=4.5 to 5.0 (kept at 60 to 80oC before use)

d. For oral route:

All species: **20%** DHE-45 suspension in 2% gelatin solution.

It is noted that caffeine, a major inactive gradient in the proposed nasal spray solution, was not present in these solutions.

2) Animals: The sponsor used 10 animals/group/sex for mice (albino, own breed,strain?, 18-29 g) and rats (albino, SIV-50 strain, 130-230 g) and 6 animals/group/sex for rabbits (mixed domestic breed, strain?, 2.17-3.67 kg).

3) Drug Treatment: Doses used in the study are summarized as follows:

a) i.v.:

* *For mice i.v.*: in mg/kg, 25, 50, 80, 100, 125, 160, 200, and 250. The injected volume was

25 ml/kg. The injected site was the animal's tail vein.

* *For rat i.v.:* in mg/kg, 80, 100, 125, 150, and 200. The injected volume and site were 10 ml/kg and the animal's tail vein, respectively.

* *For rabbit i.v.* in mg/kg, 16, 32, and 64. The injected volume was 1.0, 2.0, and 4.0 ml/kg, respectively. The injected site was the animal's ear vein.

b) oral:

In mg/kg, 2000 for mice, 2000 for rats and 1000 for rabbits. The injected volume was 25 for mice, 10 for rats and 5 ml/kg for rabbits. The drug was introduced into the animal's stomach by a tube.

c) s.c.: The solution was delivered into neck of mice or rats and lumbar region of rabbits.

* *For mice s.c.* in mg/kg, 375 and 625. The injected volume was 25 ml/kg for the LD and 12.5 ml/kg for HD.

* *For rat s.c.:* in mg/kg, 150 and 500. The injected volume was 10 ml/kg.

* *For rabbit s.c.:* in mg/kg, 15 and 60. The injected volume was 1.0 ml/kg for LD and 4.0 ml/kg for HD.

d) i.p.

For mice, the doses were 62.5, 125, 250 and 500 mg/kg and the corresponding injected volumes were 6.25, 12.5, 25 and 50 ml/kg.

4) Observations and measurements:

Mortality, latency period and signs prior to death were recorded during a 7-day observation period. The LD_{50} values were calculated according to the probit method (Miller and Tainter, Proc Soc Exp Biol Med 57: 261, 1944).

5) Postmortem: Necropsy

No data were submitted by the sponsor.

B. RESULTS:

The following tables summarize the mortality and latency period under various treatments.

a) i.v.:

** Mice:*

Dose (mg/kg)	Mortality (n/n)	Latency period
250	10/10	40 s - 60 min
200	9/10	2 min - 8 h
160	7/10	10 min - 36 h
125	1/10	40 h
100	6/10	50 min - 6 h
80	5/10	50 min - 8 h
50	0/10	-
25	0/10	-

Signs prior to death included: drowsiness, jerking, motor excitation, flaccidity and forced respiration. These drug's effects were also observed in survival animals, but subsided, however, after about 10 hours. From the data, the sponsor suggested that the **LD_{50}** and the **maximum tolerated dose (MTD)** for i.v. route in mice was **117 mg/kg and 50 mg/kg**, respectively.

** Rats:*

Dose (mg/kg)	Mortality (n/n)	Latency period
200	10/10	0 - 30 s
150	7/10	0 - 60 s
125	4/10	1 - 300 s
100	1/10	60 s
80	0/10	-

Signs prior to death included cramps, jerking, motor excitation, forced and slowed respiration. These drug's effects were also observed in survival animals, but subsided, however, after about 10 hours. From the data, the sponsor suggested the **LD_{50}** and **MTD** for rats were **130 and 80 mg/kg**.

** Rabbits:*

Dose (mg/kg)	Mortality (n/n)	Latency period
64	6/6	0 - 90 min
32	1/6	8.5 h
16	0/6	-

Signs prior to death developed directly after injection and included drowsiness, disturbance of equilibrium, motor excitation, cramps, jerking, piloerection, opisthotonos and forced breathing. These drug's effects also were observed but subsided after about 24 hr in survival animals. From the data, the sponsor suggested the **LD_{50}** and **MTD** for rabbits were **37** and **16 mg/kg**.

b) Oral:

No mortality was observed in mice (up to 2000 mg/kg), rats (2000 mg/kg) and rabbits (1000 mg/kg). The only observable sign was loss of appetite (in the rabbits) for the first 2 to 3 days.

c) s.c.

** Mice:*

No mortality was observed at doses of up to 625 mg/kg s.c. At dose 375 mg/kg, the adverse signs included drowsiness, disturbed equilibration and piloerection. These signs appeared 10 min after administration and lasted for about 12 hours. In 2 animals, skin necrosis was seen. At dose 625 mg/kg, adverse signs included motor excitation, disturbed equilibration and head scratching. These signs appeared 2 min after administration and lasted for about 24 hour.

** Rats:*

No mortality was observed at dose up to 500 mg/kg. The observable signs were, at dose 150 mg/kg, slight flaccidity, piloerection, slightly forced breathing, and skin necrosis (8/10 rats); at 500 mg/kg, drowsiness, disturbance of equilibrium, forced breathing and skin necrosis.

**** Rabbits:***

Dose (mg/kg)	Mortality (n/n)	Latency period
60	2/6	5 hr
15	0/6	-

Signs prior to death included disturbance of equilibrium, flaccidity and forced and accelerated breathing. These drug's effects also were observed but subsided after about 12 hr in survival animals. In the survival animals, white fibrinous deposit was seen around the injection site.

d) i.p.

**** Mice:***

Dose (mg/kg)	Mortality (n/n)	Latency period
500	10/10	2 - 8 h
250	9/10	4 - 26 h
125	0/10	-
62.5	0/10	-

Signs prior to death elicited around 3 min after injection and included: anaesthesia, forced respiration and dorsal decubitus. These drug's effects were also observed in survival animals, but subsided, however, after about 14 hours. From the data, the sponsor suggested that the **LD_{50}** and the **maximum tolerated dose (MTD)** for i.p. route in mice was **212 mg/kg and 125 mg/kg**, respectively.

II. DHE-45 A 26-week oral toxicity study in rats (#201-001)

A. MATERIALS AND METHODS:

1) Chemicals: DHE-45,9,10-Dihydroergotamine methanesulfonate, batch # 8612, was mixed into the feed at different concentrations.

2) Animals: Rats (. 8 wk old, 170-300 g, strain?) were divided into 4 groups for different treatments as listed in the table below.

Group	DHE-45	Number and Sex of rats
Control (K)	0	10 M + 10 F
Low-level (A)	0.004% (2mg/kg/day)	10 M + 10 F
Mid-level (B)	0.020% (11mg/kg/day)	10 M + 10 F
High-level (C)	0.100% (53mg/kg/day)	10 M + 10 F

3) Observations and measurements:

Abnormal signs or behavior were examined daily. Amounts of food intake and body weights were determined weekly. Blood sampling for hematology (10 rats/ group) were performed after

4, 13 and 26 weeks and were collected from retro-orbital venous plexus. Following hematologic parameters were measured from 5 animals/group/sex: **Hb, Hct, Erhyt, Reti, Leu, Differential count, Glucose, BUN, tot Protein, Achesterase, Chol, SGPT, SGOT, AP, Na, K, P, Lactate, Trigly, and Free Fatty A.** After 26 weeks, urinalysis was performed. Following organs were weighed: **spleen, liver, kidneys, adrenals, and testes.** Following organs were processed for histology: **heart, lung, liver, spleen, pancreas, stomach, intestines, kidneys, urinary bladder, adrenals, testes, ovary, uterus, prostate, pituitary, thyroid, parathyroid, thymus, lymph node, salivary gland, skeletal muscle, peripheral nerve, and bone marrow.** Only organs from rats group K (control) and group C (high-level) were examined histologically.

5) Necropsy

Full necropsy of all rats was performed after anesthesia and exsanguination.

B. RESULTS:

1) Mortality: All animals tolerated the dosing regimen. 1M group A and 1F group C died at the weeks 26 and 22, respectively. The causes were not drug-related.

2) Clinical Observations: Diarrhea was observed in animals groups B and C after week 5..

3) Body Weight No statistical difference in mean body weights of male rats among groups. For females, however, it appears that a dose-related decrease in mean body weights was seen (Figures 1a & b). Mean body weights of female group C decreased by ~10% in compared to that of controls (269 g vs 298 g).

4) Food Consumption: Mean food intake decreased in all treated females and the decrease was drug-related. There was no statistical difference in mean food intake among treated males, although slight decrease of the mean value in compared to the control was seen in group C (high-level) males.

5) Hematology - Coagulation: Except slight leucopenia (<9000 leukocytes/ml) observed in few animals in all rats, hematology measurements did not reveal any significant changes attributable to drug-treatment.

6) Clinical Chemistry: Major finding was increased SGPT levels in both M and F group C (mean SGPT for male = 57.6, for female=41.6 units/l) in compared to the levels of controls (mean SGPT for male=22.9, for female=23.5 units/l). In group C, highly elevated levels of SGPT were seen in 1M (rat #C_{65}, SGPT level=140 units/l)) and 1F (rat #C_{77}, SGPT level=99.4 units/l). Organ histology did not reveal equivocal abnormalities in these rats, however.

7) Urinalysis: No dose-related changes in pH, protein, glucose, ketones, blood or microscopic examinations were considered to be drug-related.

8) Necropsy: No drug-related findings were clearly observed.

9) Organ Weights: In the group C rats, there was slight decrease in mean absolute weights of the kidney in both M and F. This change, however, was equivocal.

10) Morphologic and Histologic Pathologies: No pathological changes were considered drug-related.

III. DHE-45 A 26-week oral toxicity study in dogs (#201-002)

A. MATERIALS AND METHODS:

1) Chemicals: DHE-45,9,10-Dihydroergotamine methanesulfonate, batch # 8612, was given in gelatin capsule at different doses daily, 7 days a week.

2) Animals: Twenty-four Beagles, aged 7-13 months old and obtained from different sources were divided into 4 groups for different treatments as listed in the table below.

Group	DHE-45	Number and Sex of dogs
Control (K)	0	3 M + 3 F
Low-level (A)	0.5 mg/kg/day	3 M + 3 F
Mid-level (B)	1.5 mg/kg/day	3 M + 3 F
High-level (C)	5.0 mg/kg/day	3 M + 3 F

Control (K) dogs received gelatin capsules containing 50 mg glucose. Duration of treatment was 26 weeks.

The animals were immunized with measles vaccine, Fromm DHL, and Tempacin HL2 (SHL). Vermicides were also given (piperazine, Formocibazol or/and Tenoban).

3) Observations and measurements:

Abnormal signs or behavior were examined daily. Amounts of food intake and body weights were determined weekly. Blood sampling for hematology were performed after 2, 4, 13 and 26 weeks and were collected from a superficial fore-limb vein. Following hematologic parameters were measured from: **Hb, Hct, Erhyt, Reti, Platelets, Prothrombin time, Differential count, Glucose, BUN, tot Protein, Chol, SGPT, AP, Na, and K.** After 4,13 and 26 weeks, urinalysis was performed.

Electrocardiography (standard bipolar and unipolar limb leads, and unipolar c hest leads) was performed in all animals. Following organs were weighed: **spleen, liver, kidneys, adrenals, and testes.** Eye examinations (slit lamp, retinoscopy) following mydriatic (with Mydriaticum) was performed in all dogs. Following organs were processed for histology: **heart, lung, liver, gall bladder, spleen, pancreas, stomach, intestines, kidneys, urinary bladder, adrenals, testes, ovaries, uterus, prostate, cerebral cortex, mid-brain, cerebellum, pituitary, thyroid, parathyroid, thymus, cervical and mesenteric lymph nodes, bone-marrow (femur), skeletal muscle, peripheral nerve, aorta, retina, breast and ear.** Organs from all dogs were examined histologically.

4) Necropsy

Full necropsy of all 24 dogs was performed after sacrificed by intravenous thiopentone.

B. RESULTS:

1) Mortality: All animals tolerated the dosing regimen.

2) Clinical Observations: Major findings that appear to be drug-related were: 1) Miosis: 4/6 dogs group A, 2/6 dogs group B and 5/6 dogs group C; 2) prolapse of nictitating membrane: 1/6 group A, 1/6 group B and 4/6 group C; 3) excessive salivation: at Week 10 onward, 6/6 group B and 6/6 group C.

3) Body Weight Significant decreases in mean body weight gains were observed in the mean dose group (Group C) when compared to the control means (Table below)

	Mean Body Weight (kg)		% Weight Changes	
	Males	Females	Males	Females
Controls	11.6	6.8	14.6	13.7
Group A (0.5 mg/kg)	10.3	9.2	11.3	9.2
Group B (1.5 mg.kg)	13.1	11.0	11.4	11.9
Group C (5.0 mg/kg)	12.6	8.3	2.9*	8.5*

4) Food Consumption: No changes in food intake among groups.

5) Hematology - Coagulation: Hematology measurements did not reveal any significant changes attributable to drug-treatment.

6) Clinical Chemistry: Major finding in blood chemistry was increased SGPT levels in both M and F group C (mean SGPT at Week 26 for M = 20.7 and for F=24.1 units/l) when compared to the pre-tested levels (mean SGPT for M=16.1 and F=16.9 units/l).

7) Urinalysis: No dose-related changes in pH, protein, glucose, ketones, blood or microscopic

examinations were considered to be drug-related.

8) Electrocardiography: Following major findings were observed: 1) decreased heart rate with prolong PQ intervals at Week 4 onward in all treated groups; 2) increased incidence of deep negative T waves in leads II and III at Weeks 13 and 26.

9) Eye examinations Major findings were: 1) punctate superficial corneal clouding observed in both control and treated dogs, 2) Mucous conjunctivitis in 1 dog group B and 1 dog group C. Whether these findings were drug-related are equivocal.

10) Necropsy: Following findings appeared to be drug-related and were: 1) induration and slight thickening of dependent ear margins to a depth of 1-3 mm in 5/6 dogs group B and 5/6 dogs group C; 2) pale appearance on the liver surface in 3/6 dogs group C; 3) hemorrhagic foci in urinary bladder mucosa in 3/6 dogs group C (Table 1).

10) Organ Weights: In the group B and C dogs, there were significant decreases in mean absolute and relative weights of the spleen (~ 50% reduction). In the group A and C dogs, there was slight but significant increase in mean absolute and relative weights of the heart (~ 20%).

11) Morphologic and Histologic Pathologies: Following relevant changes were observed: *1) liver*: two bile thrombi in 1 dog group A and 1 dog group C and increased lipogenic pigment in hepatocytes in 1 dog group C; *2) spleen*: decreased number of reaction centres in 1 dog group A and 3 dogs group C; *3) kidney*: cortical granulosa in 1 dog group B and 1 dog group C; *4) urinary bladder:* acute cystitis in 1 dog group A, 1 dog group B and 2 dogs group C; and *5) ear skin*: moderate to severe hyperkeratosis in 1 dog group A, 2 dogs group B and 5 dogs group C. According to the sponsor, acute cystitis was considered to be due to catheterization for urine sampling. Except the ear skin abnormality,none of other observations were considered to be drug-related.

APPEARS THIS WAY
ON ORIGINAL

RERPRODUCTIVE STUDY

(Vol. 1.6; pp. 05-00846 - 05-01353)

Following tests were conducted without GLP standards by the sponsor. Fertility study (Segment I) was perfomed in rats, peri-and post-natal toxicity study (Segment III) was performed in rats and rabbits. Teratology study (Segment II) was performed in rats and rabbits and Stumptailed Macaques.

I. FERTILITY STUDY IN RATS (Segment I):

a) Female rats:

1. Methods: Female rats (CFE-strain, albino, 200-300 g, 10 week old) were treated orally by gavage with DHE-45 (Batch No. 72007; suspended in ~ 2% gelatin solution). Three dose levels were employed: 3, 10 and 30 mg/kg/day. Each group contained 30 females. On Day 15 after treatment, 2 females were caged with one male rat for insemination. Vaginal smears were examined to confirm the insemination. From the day of coitum (Day 0 p.c.), the rat was caged singly and was continued to received the treatment for the next 21 days. Each group was divided into 2 subgroups of 15 females. One subgroup from each group was killed on Day 13 p.c. whereas those of the other were allowed to rear their young until day 21 p.c.

All females were examined macroscopically postmortum. Dams and litters were examined.

2. Results: Major findings were:

- No changes in mortality and weight gain in all mothers.
- Copulation index and fertility index did not differ from controls.
- No significant changes in litters size, litter number, or resorption rate.
- When examined on Day 21 p.p., following noticeable changes were observed:
  * Increased loss of pups in group C when compared to other groups (20% loss vs. 6-8% loss),
  * One out of ~100 pups in each treated groups showed retarded ossification of phalanges.

The above changes, however, were equivocal.

b) Male rats:

1. Methods: Male rats (CFE-strain, albino, 200-300 g, 10 week old) were treated orally by gavage with DHE-45 (Batch No. 72007; suspended in ~ 2% gelatin solution). Three dose levels were employed: 3, 10 and 30 mg/kg/day. Each group contained 15 males. On Day 64 after treatment, 1 male was caged with 2 females for a maximum period of 2 weeks. Vaginal smears were examined to determine the trace of sperms. From the day of coitum (Day 0 p.c.), the female rats were caged singly and the male rats were killed as soon as they had inseminated the females. Each group of females was divided into 2 subgroups of 15 females. One subgroup from each groups was killed on Day 13 p.c. whereas those of the other were allowed to rear their young until day 21 p.c.

All males and females were examined macroscopically post mortem. Dams and litters were examined. Following organs were examined: sex organs, liver, kidneys, adrenals, spleen, lung, heart, stomach and intestines.

2. Results: Major findings were:

- 1 male in the 10 mg/kg group died. This event was not considered to be drug-related. No other mortality was observed.
- Weight gains in all males were normal when compared to those in the controls.
- Copulation index and fertility index in the treated groups did not differ significantly from controls.
- No significant changes in litters size, litter number, mean number of corpora lutea per dam or resorption rate among groups.
- When examined on Day 21 p.p., following noticeable changes were observed:
  * Increased loss of pups in treated groups compared to controls. Folowing Table summarizes the finding:

Groups	Loss of pups (4-21 days p.p.) (% of live pups within each group)
Controls	5.1
3 mg/kg	9.3
10 mg/kg	15.7*
30 mg/kg	10.9*

* = Significantly differs from control at $p < 0.05$.,

The results, howeve, do not appear to be drug-related.

*fetal weight gains, sex ratios, and incidence of pups showing anomalities were comparable among groups.

II. TERATOLOGICAL STUDIES (Segment II)

** IN RATS:

a) Methods

Pregnant rats (CFE-strain, albino, 10 week old, 180-220 g) were treated orally by gavage with DHE-45 (Batch No. 8612; suspended in ~ 2% gelatin solution). Four dose levels were employed: 1, 3, 10 and 30 mg/kg/day. Each group contained minimum of 20 dams/dose level. Two females were caged with one male rat for insemination. Vaginal smears were examined to determine the trace of sperms. From the day of coitum (Day 0 p.c.), the inseminated females (20 rats each group) were treated with DHE-45 from Day 6 to Day 15 p.c.

All females were weighed on Day 0, 6, 15 and 21 p.c. and were examined macroscopically postmortum. On Day 21 p.c. all dams were sacrificed and examined for anomalies. The fetuses were delivered by cesarean section. All morphological anomalies of the fetuses or the youngs were grouped as: a) Type A- anomalies developed during the late fetal phase of maturation; b) Type B- anomalies occurred

during the early fetal phase of organ differentiation and fetal growth; or c) Type C- anomalies developed during organogenesis.

b) Results: (Table 2)

** Dam data*: General findings were

- All animals survived except 1 rat died in the 10 mg/kg group on Day 11 p.c.
- Pregnancy rates in all groups, ranged from 67% (in 10 mg/kg group) to 90% (in 30 mg/kg group), and were not significantly different among each others.
- No changes in mean body weight gains in all treated and control groups.

** Litter Data:* General findings were:

- No clear drug-related changes in mean number of implantation sites/litter
- The mean number of live fetuses/liter (litter size) in the 30 mg/kg group was significantly lower than the control mean (8.8 vs. 10.9).
- The mean % of prenatal mortality in the 30 mg/kg group was slightly higher than the control mean (11.9% vs. 5.5%)
- There was slight but insignificant increase in mean fetal body weight gains in all groups.
- Sex distribution was equal in all groups
- There was slightly higher Type-A anomalies in the fetuses of 10 mg/kg group, however, all values of abnormal fetuses in the treated groups were well within the norm of spontaneous changes for the species.

**** IN RABBITS:**

a) Methods

Pregnant rabbits (Swiss hare, 5-6 month old, ~3.5 kg) were treated orally by gavage with DHE-45 (Batch No. 8612; suspended in ~ 2% gelatin solution). Four dose levels were employed (1, 3, 10 and 30 mg/kg/day) for Experiment 1. Two doses levels were employed (1 and 3 mg/kg) for second experiments. Each group contained minimum of 10 dams/dose level. One female was caged with one male rabbit for insemination. Vaginal smears were examined to confirm the insemination. From the day of coitum (Day 0 p.c.), the inseminated females (10 rabbits each group) were treated with DHE-45 from Day 6 to Day 18 p.c.

All females were weighed on Day 0, 6, 18 and 29 p.c. and were examined macroscopically postmortum. On Day 29 p.c. (2 to 3 days before expected delivery) all dams and were sacrificed and examined for anomalies. The fetuses were delivered by cesarean section. All morphological anomalies of the fetuses or the youngs were grouped as: a) Type A- anomalies developed during the late fetal phase of maturation; b) Type B- anomalies occurred during the early fetal phase of organ differentiation and fetal growth; or c) Type C- anomalies developed during organogenesis.

b) Results:

** Dam data*: General findings were

-Increased number of dead was observed in treated rabbits:

Dose (mg/kg)	No of dead	
	pregnant	non-pregnant
0	0	0
1	2	0
3	0	0
10	2	0
30	4	0

- Pregnancy rates in all groups, ranged from 82% (in 3 mg/kg group) to 100% (in 10 mg/kg group), were not significantly different among each others.

- No significant changes in mean body weight gains in all treated and control groups.

** Litter Data:* General findings were:

- No clear drug-related changes in mean number of implantation sites/litter
- The mean number of live fetuses/liter (litter size) were similar in all groups.
- The mean % of prenatal mortality in the 1 and 3 mg/kg groups were slightly higher than the control mean (25% vs. 14%) in the first set of experiments, however, there were no significant differences among groups in the subsequent repeat experiments.
- There was slight but insignificant increase (1-10%) in mean fetal body weight gains in all treated groups compared to control mean. No drug-related effects were observed, however.
- Equally sex distributions in all groups were observed.
- There was slightly higher Type-A anomalies in fetuses of treated groups, however, alll values of abnormal fetuses in the treated groups were well within the norm of spontaneous changes for the species. Gallbladder aplasia, a genetically determined variation foun spontaneously in this strain, were observed in several animals in all treated and control groups.

**** IN STUMPTAILED MACAQUES:**

a) Methods

Seven female adult stumptailed macques (Macaca arctoides, age?, weighed 7.1-11.9 kg) were mated with healthy males on the optimal mating day (mid-cycle-2 days). The day of mating was considered as day 0 post coitum (p.c.). Seven pregnant females were then treated orally by gavage with 5 mg/kg/day DHE-45 (Batch No. 72006 in gelatin capsules). Four pregnant animals were treated from Day 20 to 29 p.c. and 3 animals were treated from Day 30 to 39 p.c.

On approximately Day 100 p.c., the fetuses, with placenta and membranes intact, were delivered by cesarean section. For dams, food intakes and general state of health were determined. For fetuses,

sex, weights, and anomalies were determined.

b) Results:

** Dam data*: No changes in food intakes or general state of health. What signs of state of health being considered were not specified by the sponsor.

** Litter Data:* Except 1 female fetus from treated mother had subserous haematoma on the urinary bladder which was not drug-related, no other anomalies were observed.

III. PERI- AND POSTNATAL STUDIES (Segment III)

** IN RATS:

a) Methods

Female rats (CFE-strain, albino, 180-220 g, 10 week old) were treated orally by gavage with DHE-45 (Batch No. 8612; suspended in ~ 2% gelatin solution). Two dose levels were employed: 3 and 30 mg/kg/day. Each group contained minimum of 10 dams/dose level. Two females were caged with one male rat for insemination. Vaginal smears were examined to determine the trace of sperms. From the day of coitum (Day 0 p.c.), half of the inseminated females (15 of each group) were treated with DHE-45 from Day 15 p.c. to the day of parturition. The other half of inseminated females were treated with DHE-45 from Day 15 p.c. to Day 21 post partum.

All females were examined macroscopically postmortum. Dams and litters were examined 0 p.p, 7 p.p. and 21 p.p. On Day 21 p.p. all dams and young were sacrificed and examined for anomalies.

b) Results: (Table 3)

I. Treatment from Day 15 p.c. to Day 0 postpartum:

** Dam data*: General findings were

- No mortality
- Normal pregnancy rates in all groups
- No changes in mean body weight gains in both treated groups, up to Day 0 p.p.
- Mean body weight gains in the 30 mg/kg females were significantly higher than that in control and 3 mg/kg females (11.4% vs. 3.6%).

** Litter Data:* General findings were:

- No changes in mean number of implantation sites/litter, liter size, prenatal mortality, postnatal mortality.
- No changes in mean fetal body weight gains in all groups.
- Equally sex distributions in all groups
- All values of abnormal fetuses in the treated groups were well within the norm of spontaneous changes for the species.

II. Treatment from Day 15 p.c. to Day 21 postpartum:

** Dam data*: General findings were:

- No mortality
- Normal pregnancy rates in all groups
- No changes in mean body weight gains in both treated groups, up to Day 0 p.p.
- Mean body weight gains in the 30 mg/kg females (11.4%) were significantly higher than the corresponding means in control and in 3 mg/kg groups (~ 3.6%).

** Litter Data:* General findings were:

- No changes in mean number of implantation sites/litter, liter size, and prenatal mortality.
- Slight reductions in mean % postnatal mortality in treated groups compared to controls (3-8% vs. 19%).
- No significant changes in mean fetal body weight gains in all groups.
- Approximately equal sex distribution in all groups
- All values of abnormal fetuses in the treated groups were well within the norm of spontaneous changes for the species.

**** IN RABBITS:**

a) Methods

Female rabbits ('yellow-silver' strain, 5-6 mo old) were treated orally by gavage with DHE-45 (Batch No. 8612; suspended in ~ 2% gelatin solution). Two dose levels were employed: 3 and 30 mg/kg/day. Each group contained minimum of 15 dams/dose level. One female was caged with one male rabbit for insemination. Vaginal smears were examined to determine the insemination. From the day of mating (Day 0 p.c.), half of the inseminated females (15 of each group), caged singly, were treated with DHE-45 from Day 18 p.c. to the day of parturition (Day 29 p.c. or Day 0 p.p.). The other half of inseminated females were treated with DHE-45 from Day 18 p.c. to Day 21 post partum.

All females were examined macroscopically postmortum. Dams and litters were examined 0 p.p, 7 p.p. and 21 p.p. On Day 21 p.p. all dams and young were sacrificed and examined for anomalies. All morphological anomalies of the fetuses or the youngs were grouped as: a) Type A- anomalies developed during the late fetal phase of maturation; b) Type B- anomalies occurred during the early fetal phase of organ differentiation and fetal growth; or c) Type C- anomalies developed during organogenesis.

b) Results:

1. Treatment from Day 18 p.c. to day of parturition:

** Dam data*: General findings were

- No mortality
- Normal pregnancy rates in all groups
- No obvious changes that were considered drug-related in mean body weight gains in both treated groups, up to Day 0 p.p.
- Slight decreases in % mean weight gains in treated groups in compared with %

controls. These changes, however, were not drug-related.

* *Litter Data:* The sponsor reported that 6 pups of one litter from the low dosage group (3 mg/kg) had to be sacrificed prematurely (at Day 18 p.p.) because of inflammation of the maternal animals mammary glands found later. This was considered an isolated case and non-drug-related. General findings were:

- No drug-related changes in mean number of implantation sites/litter, liter size, prenatal mortality, postnatal mortality .
- No drug-related changes in mean fetal body weight gains in all groups.
- A preponderance of male in all 3 groups but equal distributions in all groups
- All values of abnormal fetuses in the treated groups were well within the norm of spontaneous changes for the species.

II. Treatment from Day 18 p.c. to Day 21 postpartum:

* *Dam data*: General findings were:

- No mortality
- Normal pregnancy rates in all groups
- No drug-related changes in mean body weight gains in both treated groups, up to Day 21 p.p.

* *Litter Data:* General findings were:

- No drug-related changes in mean number of implantation sites/litter, liter size, and prenatal mortality.
- No significant changes in mean fetal body weight gains in all groups.
- Approximately equal sex distributions in all groups
- All values of abnormal fetuses in the treated groups were well within the norm of spontaneous changes for the species.

APPEARS THIS WAY
ON ORIGINAL

EVALUATION AND RECOMMENDATION

I. SUMMARY AND EVALUATION:

General Background: Dihydroergotamine mesylate has been marketed (DHE 45®) as a sterile solution for i.v. and a.m. injections for the treatment of migraine headache. Dihydroergotamine is an α-adrenergic blocking agent and is well known for its pharmacological effects of constricting peripheral and cranial blood vessels. The compound, likes other ergot alkaloids, also has the properties of serotonin agonism and antagonism. It is the interaction of the ergots, including DHE, to various serotonin receptors, particularly the 5-HT_{1d} receptor, appears to be the main mechanism of action for the anti-migraine effect. The drug, DHE-45, has very low oral bioavailability (<1%), due to both poor permeation across the gastrointestinal mucosa and a high presystemic clearance due to hepatic metabolism. The sponsor has proposed the nasal route of administration as way of increasing systemic availability of an alternative to parenteral administration. The nasal passages provide a highly vascularized structure for absorption (total surface area in man ~ 180 cm^2). Studies of pharmacokinetics and metabolism of the drug, using intranasal route in man and rat, provided the following informations: *1)* the drug is rapidly absorbed in a dose-dependent manner (t_{max} = 15 - 45 min), *2)* compared to the i.v. or i.m. route of administration, the bioavailability of D.H.E. 45 nasal spray is 30-40%, *3)* the total body clearance is approximately 1.5 L/min which reflects mainly hepatic clearance, 4) biliary excretion was found to be the predominant pathway of secretion, and *5)* the plasma half life approximates 10 hr in man and rats. According to the sponsor, caffeine (10 mg) is added to the drug formulation to enhance the solubility of the drug and act as a stabilizer. Caffeine at doses of 100 - 150 mg p.o., however, acts as a CNS stimulant and a cerebral blood vessels vasoconstrictor (Goodman & Gilman, in *Pharmacol. Basis for Therapeut.* 1990).

Species	Routes	LD_{50}	MTD (mg/kg)
Mice	i.v.	117	50
Rats	i.v.	130	80
Rabbits	i.v.	37	16
Mice	s.c.	>625	-
Rats	s.c.	>500	-
Rabbits	s.c.	~60	-
Mice	i.p	212	125
Mice	oral	>2000	-
Rats	oral	>2000	-
Rabbits	oral	>1000	-

Toxicology: Varous preclinical toxicity studies have been conducted by the sponsor. None of

these studies, however, were done under GLP standards. Acute toxicity tests in mice, rats and rabbits were conducted. The LD_{50} and MTD levels for each species in various routes of administration are summarized in the Table above. The results of these studies showed that oral absorption of the drug was poor. Major adverse signs included ptosis, sedation, hypokinesis and forced respiration. In surviving animals these adverse signs generally subsided after ~ 24 hr. It can be seen from the Table that the LD_{50} of DHE-45 via i.v. administration in mice, rats and rabbits approximate 1500, 1600 and 462 times, respectively, the maximum recommended daily dose based on a 50 kg man.

Chronic oral toxicity studies (26-weeks) in rats and dogs were performed by the sponsor. In rats, 3 oral doses were employed: 2, 11 and 53 mg/kg/day. It appears that the non-observable adverse effect level (NOAEL) was 11 mg/kg/day (approximately 138 times the maximum recommended daily dose for 50 kg man). At dose of 53 mg/kg/day (approximately 660 times the maximum recommended daily dose for 50 kg man), major adverse effects were slight reduction in mean body weight gains (10%) and increased plasma SGPT levels (~ 2-fold). There were no obvious changes in organ weights or organ histopathologies. In dogs, 3 oral doses were employed: 0.5, 1.5 and 5 mg/kg/day. It appears that the NOAEL level was 1.5 mg/kg/day (approximately 19 times the maximum recommended daily dose for 50 kg man). At dose of 5 mg/kg/day, major adverse events were excessive salivation, prolapse of nictitating membrane, miosis, slightly decreased mean body weight gains (~10%), slightly increased plasma SGPT levels (~ 50%) and decreased heart rate with prolong PQ intervals. The only noteworthy necropsy finding was the thickening and induration of the ears of these animals. This moderate to severe hyperkeratosis at the ears was attributable to a vasoconstrictive action of the drug. The plasma levels of D.H.E.-45 corresponding to the oral doses employed in both rat and dog toxicity studies were not provided by the sponsor.

Reproductive toxicity studies employing dose levels of 1, 3, 10 and 30 mg/kg/day orally (approximately 12, 36, 120 and 360 times the maximum recommendfed daily dose based on a 50 kg man).were performed in rats, rabbits, and monkeys. Its finding was that DHE-45 did not produce any evidence of adverse reproductive effects. It is noteworthy that oral administration of DHE-45 up to 30 mg/kg/day to female and male rats and rabbits did not produce any observable adverse effects (e.g. reduced body weight gain). Since the plasma levels of DHE-45 in these animals were not determined, it is not known whether these animals were adequately exposed to the drug levels.

In summary, the preclinical toxicity data provided here ***do not*** support the proposed clinical studies because 1) the route of administration of DHE-45 was not intranasal; 2) none of these studies followed GLP guidelines, and 3) caffeine, an excipient in the proposed clinical studies, was not included in the submited preclinical studies. However, it is noted that:

1) Preclinical toxicity studies of DHE-45 using nasal route have been performed by the sponsor in rabbits (7 days ocular irritation study) and monkeys (13-week intranasal toxicity study) (see IND #

. The only noticable effect was that at 4 mg/ml intranasally, DHE-45 caused focal irritation and/or

ulceration of the nasal mucosa in the treated animals.

2) Caffeine level, used in the proposed intranasal route (e.i., 10 mg), approximates *10-fold lower* than the level at which the cardiovascular and neuronal excitability effects was observed in human (Goodman and Gilman, Pharma. Basic Thera., 1990).

3) Preclinical toxicity studies in rats and dogs submitted here were conducted before 1979, the year when GLP became officially effective.

4) Human studies (Phases I and II) with DHE-45 using intranasal route have been conducted. Side reactions generally were considered mild and included dizziness, moderate numbness of nose and burning nasal mucosa, and sneezing and nausea.

II. RECOMMENDATION:

The sponsor should repeat the preclinical reproductive studies of DHE-45 using intranasal administration.

Kishena C. Wadhwani, Ph.D.

cc
NDA (#20-148)
Div. File / HFD - 120
K.C. Wadhwani / John Jessop/ G. Fitzgerald / Don Grilley

Fig. 1

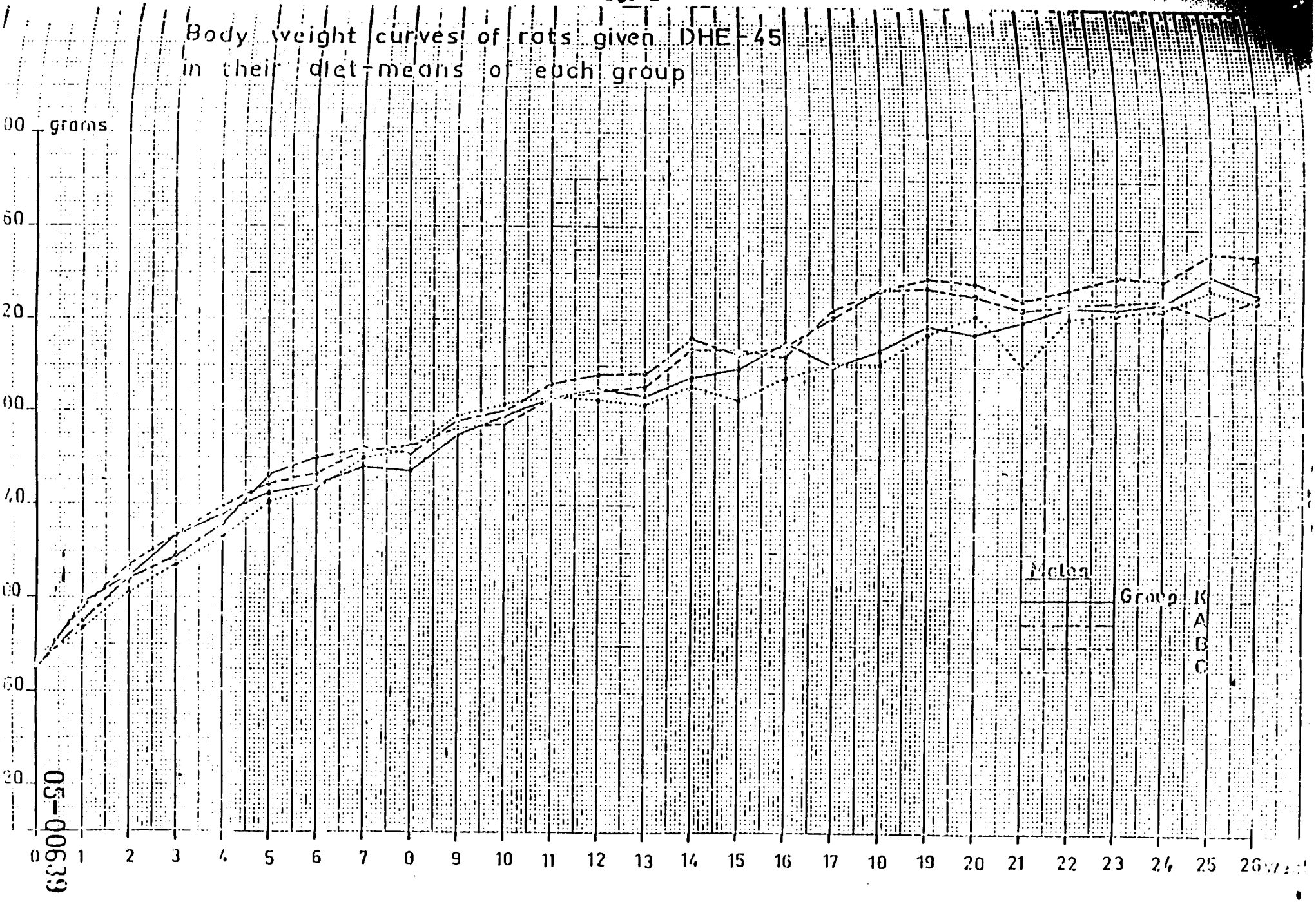
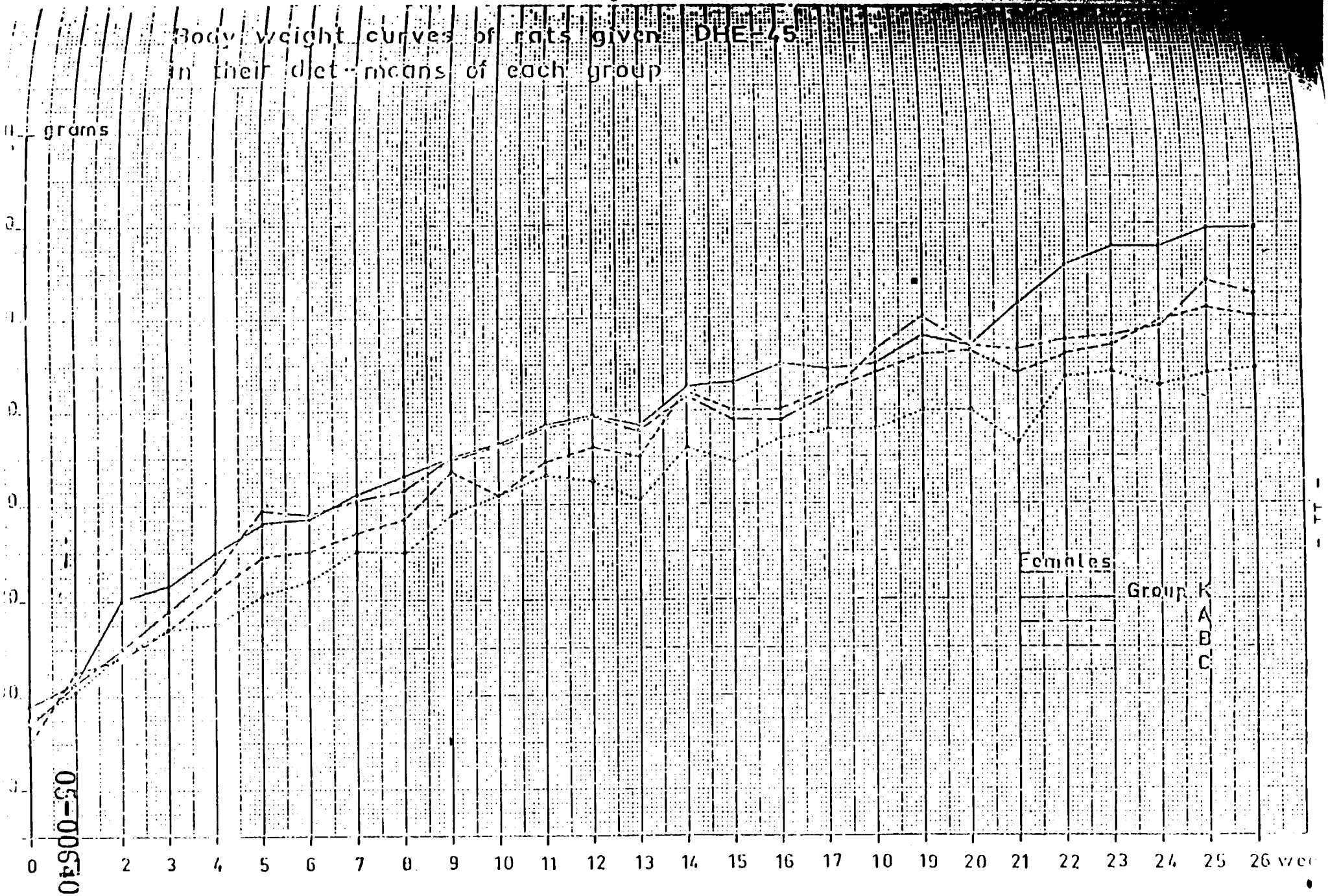


Fig 6

Fig. 2



05-0540

Table (1)

Necropsy – DOGS

DHE-45 ADMINISTERED ORALLY FOR 26 WEEKS

KEY: 0 = No change
1 = slight change
2 = moderate change
3 = marked change

	Control group				Low-level group 0.5 mg/kg/day				Mid-level group 1.5 mg/kg/day				High-level group 5.0 mg/kg/day			
	males			females	males			females	males			females	males			females
DOG NUMBERS	1	2	3	4 5 6	7	8	9	10 11 12	13 14 15			16 17 18	19 20 21			22 23 24
Thymus involution	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	0 1 0
Pneumonic foci	0	0	1	2 0 0	0	1	0	2 0 0	0	1	0	0 1 2	0	0	0	0 0 0
Liver pallor	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	0 0 0	1	0	1	0 0 1
Splenic capsule scarred	0	0	2	0 0 0	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	0 0 0
Intestinal nematodes	0	0	1	0 0 2	0	0	0	1 0 0	0	1	0	0 1 1	0	2	1	0 1 1
Intestinal tape-worms	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	0 0 0	2	0	0	0 0 0
Renal subcapsular white spots	0	0	0	0 2 0	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	0 0 0
Urinary bladder mucosa-hemorrhagic foci	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	0 0 0	1	1	0	.0 0 2
Testes small	0	0	0	- - -	0	0	0	- - -	0	0	0	- - -	0	0	1	- - -
Aorta - at eroma above aortic valves	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	0 0 0	1	0	0	0 0 0
Internal Hydrocephalus	0	0	0	0 0 0	0	0	0	0 0 0	0	0	0	2 0 0	0	2	0	0 0 0
Liver margin induration	0	0	0	0 0 0	0	0	0	0 0 0	2	1	2	2 2 0	2	2	2	2 1 0

Table (2)

Teratology in rats

embryolethal nor [illegible]

Summary of experimental data

Dose mg/kg (1)	Mortality %	Pregnancies %	Corpora lutea/ litter	Implantations /litter	Live fetuses /litter	Live fetuses % (2)	Prenatal Mortality % (2)	Abnormal fetuses % (3)
0 (14)	0	86	11.6	9.3	8.0	85.7	14.3	0
1 (18)	11	94	11.6	8.9	6.3	70.1	29.9*	0.92
3 (17)	6	82	11.5	9.0	6.7	74.4	25.6*	0
10 (15)	13	100	11.1	8.6	7.9	92.0	8.0	0.96
30 (17)	22	94	9.9	8.6	7.0	81.6	18.4	1.16
0 (15)	13	80	10.1	9.0	7.9	87.7	12.3	0
1 (18)	6	83	10.9	8.6	7.2	84.2	15.8	0.96
3 (16)	6	94	9.3	8.3	7.2	86.0	14.0	0

(1) No. of animals in brackets
(2) Percent of implantations
(3) Type-C anomalies in % of all living and dead fetuses examined
* $P < 0.05$

CONTROL

Mon Sey

Table 2 Data from 22 control pregnancies in Macaca arctoides

Mother		Fetus				Findings
No. ♀	Parity	C.section days p.c.	Sex	Body weight (g)	C-R Length (mm)	
141	1	100	♂	118	128	none
383	1	101	♂	139	142	none
386	1	102	♀	135	130	none
387	1	-	-	-	-	abortion day 54
389	1	99	♀	75	109	Placenta marginata, Amniotic fluid greenish brown, Fetus small-for-date
420	1	102	♂	157	133	none
425	1	103	♀	136	125	none
428	1	100	♀	112	122	none
428	6	101	♂	128	139	none
428*	7	-	-	-	-	abortion after day 50 p.c.
434	1	107	♂	163	129	none
436	1	100	♂	131	131	none
437	6	-	-	-	-	abortion day 65 p.c.
439	1	102	♂	138	136	none
440	1	99	♂	118	125	none
441	1	100	♂	108	124	none
532	6	-	-	-	-	abortion day 31 p.c.
540*	4	101	♂	110	132	none
702	2	-	-	-	-	abortion day 54 p.c.
702	3	99	♂	96	125	none
702*	5	-	-	-	-	abortion after days 67 p.c.
748	4	101	♂	100	120	none

Treated

Table 1 DHE 45 given to pregnant Macaca arctoides

Mother				Fetus				Findings
No. ♀	Parity	Dose mg/kg/day	treatment days p.c.	C.section days p.c.	Sex	Body weight (g)	C-R Length (mm)	
538	4	5	20-29	101	♀	99	119	none*
697	4	5	20-29	100	♂	124	128	none
894	1	5	20-29	102	♂	129	137	none
903	2	5	20-29	101	♂	142	139	none
746	3	5	30-39	100	♀	98	119	none
748	5	5	30-39	102	♀	106	127	none
755	3	5	30-39	101	♀	99	125	none

*subserous haematoma on the urinary bladder caused by manipulation during cesarean section.

C-R = Crown-Rump

Rearing of Rats
DHE 45
day 15 p.c. - 21 p.p.

Table II/6: Sex Distribution of Young
a) absolute figures

dose	day 0 p.p.			day 7 p.p.			day 21 p.p.		
	Young	♂	♀	Young	♂	♀	Young	♂	♀
Controls	149	81	68	141	74	67	121	61	60
3 mg/kg	100	41*)	59*)	99	40	59	97	40	57
30 mg/kg	126	67	59	120	63	57	115	59	56

*) $P < 0.05$

b) Percentage and ration ♂ : ♀

dose	day 0 p.p.			day 7 p.p.			day 21 p.p.		
	% ♂	% ♀	♂: ♀	% ♂	% ♀	♂ : ♀	% ♂	% ♀	♂ : ♀
Controls	54.4	45.6	1.19	52.5	47.5	1.10	50.4	49.6	1.02
3 ,g/kg	41.0	59.0	0.69	40.4	59.6	0.68	41.2	58.8	0.70
30 mg/kg	53.2	46.8	1.14	52.5	47.5	1.11	51.3	48.7	1.05

Table II/7: Young with anomalies

Dose	inspected young	with anomalies	
		absol.Number	% of insp. young
Controls	152	2	1.32
3 mg/kg	108	0	0.00
30 mg/kg	142	2	1.41

Table 2(1)

Table II/6 : Sex Distribution of Young

a) absolute figures

Dose	day 0 p.p.			day 7 p.p.			day 21 p.p.		
	Young	♂	♀	Young	♂	♀	Young	♂	♀
Controls	38*	18	19	32	15	17	31	14	17
3 mg/kg	61**	29	24	27	18	9	27	18	9
30 mg/kg	37	20	17	29	16	13	29	16	13

* 1 young devoured
** 8 young devoured

b) Percentage and ratio ♂ : ♀

Dose	day 0 p.p.			day 7 p.p.			day 21 p.p.		
	% ♂	% ♀	♂:♀	% ♂	% ♀	♂:♀	% ♂	% ♀	♂:♀
Controls	48.6	51.4	0.94	46.9	53.1	0.88	45.2	54.8	0.82
3 mg/kg	54.7	45.3	1.20	66.7	33.3	2.00	66.7	33.3	2.00
30 mg/kg	54.1	45.9	1.17	55.2	44.8	1.23	55.2	44.8	1.23

Peri - post natal study in rabbits

Table II/7 : Young with anomalies

Dose	inspected young	Fetuses with anomalies					
		A 1)		B 1)		C 1)	
		abs.	%	abs.	%	abs.	%
Controls	37	4	10.81	2	5.41	0	0
3 mg/kg	65	15	23.07	3	4.62	1	1.53
30 mg/kg	39	4	10.25	2	7.69	0	0

1) Type A = anomalies developing during the late fetal phase of maturation.

Type B = anomalies developing during the early fetal phase of organ differentiation and fetal growth.

Type C = anomalies developing during the organogenetic phase of gestation.

Peri & post natal study in Rabbits

E. Description of Anomalies

I. Treatment from day 18 p.c. to term

	Control	3 mg/kg	30 mg/kg
A. Anomalies			
Sternebrae: 5 rudimentary	4	2	3
5 missing	1	0	0
3 misshaped	0	0	1
B. Anomalies			
Vertebral bodies fused	1	0	0
Ribs thickened	1	1	6
Sternebrae fused	1	1	1
C. Anomalies			
Encephalocele, unilateral anophthalmia and abnormal formation of nose and mouth.	1	0	0

Peri & post natal study in rabbits

E. Description of Anomalies

II. Treatment from day 18 p.c. to day 21 p.p.

	Control	3 mg/kg	30 mg/kg
A. Anomalies			
S Sternebrae: 5 rudimentary	3	10	4
5 misshaped	0	1	0
5 missing	0	4	0
Rib shortened	1	0	0
B. Anomalies			
Ribs thickened	2	3	2
Sternebrae fused	0	0	1
C. Anomalies			
Unilateral microphthalmia	0	1	0

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original Review

John J. Jessop, Ph.D., Pharmacologist

NDA#: 20-148

Review Date: March 17, 1995

Date of Submission: May 1, 1991

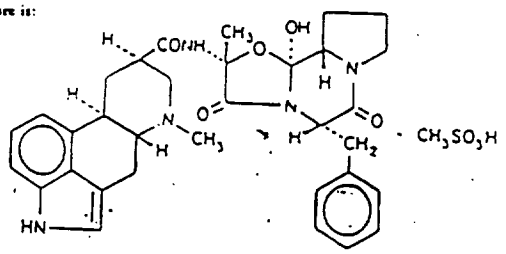
Safety Review Date: March 24, 1995

Sponsor: Sandoz Pharmaceuticals Corporation
59 Route 10
East Hanover, New Jersey 07936-1080

Drug: D.H.E. 45® (Dihydroergotamine Mesylate, (USP) Nasal Spray)

Structure:

The Chemical Structure is:



Chemical Name: Ergotamine-3', 6', 18-trione, 9, 10-dihydro-12'-hydroxy-2'-methyl-5'-(phenyl-methyl)-,(5'∝)-monomethanesulfonate.

Molecular Formula: $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$

Molecular Weight: 679.79

Pharmacological Category: Synthetic Ergot Alkaloid

Indication: Symptomatic treatment of common of classic migraine headaches in adults

Related INDs/NDAs:

Proposed Clinical Use:

The proposed clinical use of DHE 45 is as follows: Initially, the proposed dose is one puff from intranasal delivery system to each nostril (0.5 mg DHE and 1.25 mg caffeine per puff, total dose 1 mg DHE for 2 nostrils). This dose can be repeated, if necessary, in 15 minutes. The proposed maximum total dose per headache is 2 mg DHE and 5 mg caffeine. The proposed maximum total dose per 24 hour period is 4 mg DHE and 10 mg caffeine. The proposed maximum dose per week is 12 mg DHE and 30 mg caffeine.

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original Review

John J. Jessop, Ph.D., Pharmacologist

NDA#: 20-148

TABLE OF CONTENTS

Studies Reviewed in This Submission :

Toxicology

Acute

1. Acute toxicity study of dihydroergotamine or DHE-45 nasal spray solution administered by the oral and intravenous routes to rats and mice, (Doc. No. 203-003, Oct. 20, 1982).

2. Acute toxicity study in mice, rats and rabbits by I.V., oral, subcutaneous and I.P. routes. (Doc. No. 201-007, Oct. 18, 1971).

Subacute/Chronic/Carcinogenicity

1. 13-week toxicity study of dihydroergotamine administered by the intranasal route to Cynomolgus monkeys, Histological study to assess local tolerance by the nasal mucosa (Doc. No. 203-005, March 15, 1984).

2. 13-week toxicity study of dihydroergotamine administered by the intranasal route to Cynomolgus monkeys. Pharmacokinetics assessment. (Doc. No. 203-008, June 26, 1986).

3. DHE-45: 17-day intranasal and oral pharmacokinetics and toxicity study in the Cynomolgus monkey (Doc. No. 203-007, Oct. 25, 1983).

4. DHE: 13-week intranasal toxicity study in the Cynomolgus monkey. (Doc. No. 203-006, May 31, 1984).

5. Dihydroergotamine (DHE 45)- A 26-week oral toxicity study in rabbits. (Doc. No. 201-001, Dec 30, 1969).

6. Dihydroergotamine (DHE-45)- A 26-week oral toxicity study in dogs. (Doc. No. 201-002, Dec. 31, 1969).

7. 4-week intranasal toxicity study in rats, Vol. 5.1, 7/14/94.

8. 4-week intranasal dose range-finding toxicity study in mice, Vol. 5.1, 7/14/94.

Genetic Toxicology

1. DHE-45 (3080)--Mutagenicity evaluation using Salmonella Typhimurium (Doc. No. 201-010, June 6, 1983), Vol. 6.

2. DHE-45 (3080)--Micronucleus test for mutagenicity potential in mice (Doc. No. 201-011, June 13, 1983), Vol. 6.

3. DHE-45 (3080)--Micronucleus test and cytogenetic analysis of Chinese Hamster bone marrow cells for evaluation of mutagenic potential (Doc. No. 201-013, Sept. 16, 1985), Vol. 6.

4. Chromosomal aberrations in cells of Chinese Hamster Cell Line V79 (Doc. No. 203-127, Oct. 22, 1986), Vol. Amendment 1/26/95.

5. Test for the Induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures (Doc. No. 203-111, June 9, 1988), Vol. Amendment 1/26/95.

6. Mutagenicity evaluation in V79 Chinese Hamster Cells (HGPRT-test) (Doc. No-203-17, Oct. 5, 1990), Vol. Amendment 1/26/95.

Reproductive Toxicology

1. DHE-45-Fertility study (Segment I) in female rats, oral route (Doc. No. 201-008, March 11, 1974).

2. DHE-45-Fertility study (Segment I) in male rats, oral route (Doc. No. 201-009, March 11 1974).

3. Perinatal and Postnatal study (Segment III) in rats, oral gavage (Doc. No. 201-003, June 19, 1970).

4. Perinatal and Postnatal study (Segment III) in rabbits, oral gavage (Doc. No. 201-004, Dec. 22, 1970).

5. DHE-45-A teratological (Segment II) study in rats, oral (Doc. No. 201-005, Sept. 13, 1971).

6. DHE-45-A teratological study (Segment II) in rabbits, oral (Doc. No. 201-006, Sept. 13, 1971).

7. DHE-45 (Dihydroergotamine)-A teratological study in Stumptailed Macaques, oral (Doc. No. 201-012, Aug. 5, 1976), Vol. 6.

8. 3-month intranasal maximum tolerated dose study in rats, Vol. 6.1, 8/31/94.

9. 3-month intranasal maximum tolerated dose study in mice, Vol. 6.1, 8/31/94

10. Dose range-finding study on D.H.E. 45 nasal spray in pregnant rabbits, intranasal administration, Vol. 5.1, 7/14/94.

11. Dose range-finding study on D.H.E. 45 nasal spray in pregnant rats, intranasal administration, Vol 5.1, 7/14/94.

12. Investigation of teratogenic potential (Segment II) of D.H.E. 45 nasal spray in rabbits, Vol 5.1, 7/14/94.

13. D.H.E. 45 nasal spray: An intranasal teratology (Segment II) study in rats, intranasal administration, Vol.5.2, 7/14/94.

14. Segment I and Segment III studies in rats and mice by the intranasal route have been promised.

Pharmacology

Dihydroergotamine is an ergot alkaloid of mixed biological activity that produces vasoconstriction of peripheral and cranial blood vessels, a direct stimulatory effect on the smooth muscle, alpha adrenergic blocking activity, and depression of central vasomotor centers. DHE is a partial agonist and antagonist on serotonin receptors (activity on smooth muscles), a nonselective antagonist of dopaminergic receptors (activity on the sympathetic ganglia) and through it interactions with ∝-adrenergic receptors it is a partial agonist in veins and an antagonist in blood vessels, various smooth muscles and peripheral and central nervous systems (Goodman and Gilman, "The Pharmacological Basis of Therapeutics", Eighth Edition). Although the etiology of migraine is complex and somewhat poorly understood, it is thought that the vasoconstrictive properties of DHE are responsible for its efficacy in treating migraine, although its antimigraine effects might also be tied into its interaction with the 5-HT receptor, 5-HT_{1d} in particular. Dihydroergotamine mesylate is already approved for treatment of migraine by the Intravenous and intramuscular routes of administration. Intravenous dosing involves administration of 2 mg, with no more than 6 mg being given parenterally in one week.

Owing to an extensive first-pass hepatic metabolism, DHE is known to have very low bioavailability by the oral route. Caffeine apparently enhances the action of other ergot alkaloids, such as ergotamine, administered orally for the treatment of migraine. This is thought to be due to an increase in the oral absorption of the drug.

Toxicity of the ergot alkaloids is a concern mainly due to their effects on the uterus and the cardiovascular system. All the natural alkaloids of ergot in general are known to increase the motor activity of the uterus. DHE is listed in Goodman and Gilman (Eighth Edition) as "active on pregnant human uterus", and is considered an abortifacient at the appropriate dose. The previously approved intravenous formulation for DHE was listed as "Pregnancy Category X", meaning the drug is contraindicated for women who are, or may become pregnant.

Effects of ergot alkaloids on the cardiovascular system are complex, and ergotamine, for example, produces a slowly progressing increase in peripheral vascular resistance that persists for up to 24 hours. However, according to Goodman and Gilman (Eighth Edition), DHE has relatively little capacity to produce such effects in man. However, at higher plasma concentrations achieved by I.V. administration, DHE can cause a rapid increase in blood pressure that apparently dissipates in a few hours (Andersen et al., *Stroke* 18:120-123, 1987. Unlike some of the other ergot alkaloids, DHE is known to have a fairly low emetic potency.

ADME

1. Review of PK Information in Scientific Publication submitted with the NDA (Lau et al., Pharmacokinetics of intranasally-administered dihydroergotamine in the rat, Pharmaceutical Research, 11(11):1530, 1994).

DHE and ^{3}H-DHE were prepared as the methane-sulfonate (mesylate) salt and dissolved into an aqueous solvent containing 50 mg dextrose and 10 mg caffeine per ml of water. 24 Sprague Dawley rats received a 100µl aliquot of drug) 0.343 mg equivalent to 0.4 mg of the methanesulfonate salt) of the dose solution administered either I.V. or intranasal. Animals receiving intranasal administration were administered 25µl per nostril twice with a 250µl Hamilton Syringe with small piece of PE tubing at the end. After each dosing session, the exterior nasal region of each animal was cleaned using a piece of gauze to collect any dose that was sneezed out or leaked from the nostrils. These nasal swabs were retained for radioactive counting. Blood was sampled by tail-vein at various times up to 96 hours after drug administration, with a sufficient number of points being collected in the first 12 hours.

Following **I.V. administration**, the majority of the radioactivity was found in the **feces (79%)**, with approximately **13%** recovered in the **urine**. Similarly, **fecal elimination** was the major route of

excretion in rats receiving the **intranasal doses (73%),** compared to only about **8%** in the **urine.** Elimination of the drug by both routes was rapid and essentially complete by 48 hours post-dose. In **bile-duct cannulated rats** receiving the I.V. dose, **81% of radioactivity** was found in the **bile** at 72 hours post-dose, with the majority of excretion occurring in the first 24 hours. **Urinary excretion** accounted for **19%** of the dose. Following **intranasal administration, 24% and 16%** of the dose was recovered in the **bile and the urine**, respectively. About 47% was recovered in the feces at 72 hours.

Table 1. Pharmacokinetics Parameters of ^{3}H-DHE Following Intranasal and I.V. Administration

Route of Administration	K Parameters	Radioactivity		Plasma
		Blood	Plasma	DHE
Intranasal	t_{max1}* (h)	0.25 ± 0	0.25 ± 0	1.0
	t_{max2} (h)	2.1 ± 1.3	2.3 ± 1.2	2.0
	C_{max1} (ng.equiv/ml or ng/ml)	28 ± 4	23 ± 2	18
	C_{max2} (ng.equiv/ml or ng/ml)	28 ± 6.8	25 ± 5.5	23
	$AUC_{0-\infty}$ (ngEq.h/ml or ng.h/ml)	370 ±51	360 ± 21	170
Intravenous	$AUC_{0-\infty}$ (ngEq.h/ml or ng.h/ml)	630 ± 140	690 ± 110	420

*1=after first 25µl drug in each nostril, 2=after second 25µl drug in each nostril

With respect to absorption, the following figures apply:

1. Calculated comparing total radioactivity data in blood or in plasma following intranasal and I.V. doses, absorption=52-59%.
2. From the relative amount excreted in the urine, the fraction of intranasal dose absorbed was about 60%.
3. Using the sum of urinary and biliary excretion data from bile-duct cannulated rats, the fraction of dose absorbed was about 45%.

Therefore the fraction of intranasal dose absorbed was approximately 45-60%.

With respect to bioavailability of the intranasal dose, using the ratio of $AUC_{0-\infty}$ of plasma DHE following intranasal and I.V. dosing, the bioavailability of the parent drug was about 40%. This is similar to findings in humans that showed rapid absorption of DHE after intranasal administration, with relative bioavailability of approximately 38%.

The bioavailability estimates are somewhat lower than the estimated extent of absorption, which may be explained by a possible first-pass effect metabolism in the nasal mucosa, which is known to exhibit activities for oxidative drug metabolism. Furthermore, following both intranasal and I.V. dosing, plasma concentrations of radioactivity were substantially higher than unchanged DHE concentration (see Table 1 above) after 4 hours post-dose,probably due to circulating metabolites which exhibit longer residence times than the parent drug.

Finally, about 50% of the dose was found in the feces following intranasal administration, suggesting that at the dose volume used in this study (25µl/nostril) about half of the administered dose was swallowed into the gastrointestinal tract. Bile-duct cannulated animals revealed that biliary excretion was the predominant pathway of excretion.

Reviewer's Comment: What are these metabolites? Are they active? The sponsor has not attempted to identify the metabolites nor to determine if they have any biological activity.

2. DHE 45: 17 day intra-nasal an oral pharmacokinetics and toxicity study in the Cynomolgus monkey. No GLP Statement. Sandoz Doc. No. 203-007, October, 1983.

The purpose of this study was to evaluate the bioavailability and toxicity of DHE 45, administered intranasal, in the Cynomolgus monkey, and to select a suitable dose for a subsequent 13 week intra-nasal study.

Study Description

Test article

Ten 9 mg tablets of DHE 45 and DHE 45 Nasal Spray Batch number G005, 4 mg/ml DHE 45.

Animals

Two wild caught Cynomolgus monkeys (Macaca fascicularis), 2.2-2.6 kg.

Route of Administration

The drug was administered intranasal as the spray form using modified human applicators, and orally by tablet.

Dose levels, frequency and duration of administration

Table 3.2.3 Dose levels, frequency and duration of administration

Day of study	Route	Dosage	
		Animal 996M	Animal 997M
1	Oral	One 9 mg tablet	One 9 mg tablet
2-7	N/A	Undosed	Undosed
8	Nasal	2 pulses	4 pulses on 3 occasions
9-17	Nasal	4 pulses on 3 occasions each day	

Each pulse administered 0.115 ml of DHE 45 formulation equivalent to 0.46 mg of dihydroergotamine as the methane sulfonate.

Pharmacokinetics studies

Blood samples (2.5 ml) were taken from both animals immediately before dosing and again 1, 2, 4, 6, 8, and 24 h after dosing on each of days 1, 8 and 17. Twenty-four hour urine samples were also collected.

Observations

Clinical symptoms, body weight, food consumption, histopathology (tissue samples were taken but not evaluated).

Results

Mortality

Animal 997 died on the morning after its final dose. The previous day it was observed to have very little strength in its legs and it collapsed on return to its cage after being bled 8 hours after dosing. No cause of death was determined, but at necropsy pallor of the liver and heart were noted.

Samples of liver and gastro-intestinal tract were tested for aerobic and anaerobic bacteria but pathogenic bacteria could not be isolated.

Clinical signs .

No clinical signs other than the weakness in the animal that died were observed.

Body weight and food consumption

Both animals lost weight (M996-150g; M997-50g) 24 hours after oral dose of 9 mg DHE, which was associated with a marked decrease in food intake on Day 1 for both animals. During the subsequent period off dose the animals gained back the majority of that lost weight.

Further marked weight loss (M996-200g; M997, 100g) was noted on the first day of the intranasal dosing (Day 8), and was again associated with a loss in appetite. No further weight loss occurred after this time.

Toxicokinetics

These data were found in Volume 4, Doc. No. 203-008, page 05-00363.

Assay of DHE in plasma and urine was accomplished through the use of two different radioimmunoassays (RIA), one with an antibody specific for the peptide portion of DHE to detect the parent drug and a second RIA to measure the metabolites.

Parent drug $t_{1/12}$ = 6 hours. T_{max} was 1-2 hours by both routes of administration, indicating fairly rapid absorption. AUCs and metabolic ratio of parent drug:parent drug + metabolite are shown in the following Table:

The mean area under the plasma concentration/time curves (AUC), calculated for doses of 9 or 6 mg/animal and for 1 mg/kg

Route	Dose	AUC $_{0\text{-}24}$ (ng.h/ml) Parent drug	AUC $_{0\text{-}24}$ (ng.h/ml) Parent drug + metabolite	Metabolic ratio Parent drug/ Parent drug + metabolite
Oral	9 mg/animal	17.25	88.11	20%
	1 mg/kg	4.74	23.72	20%
Intranasal	6 mg/animal	96.08	134.30	71%
	1 mg/kg	39.88	56.18	71%

The ratio of AUC for the parent drug over the AUC for the parent + metabolites was 20% and 70%, respectively after administration by the oral and the nasal route. Thus, the proportion of parent drug in the plasma after intranasal administration was higher than after oral administration, probably because by the intranasal route one avoids the hepatic first-pass effect.

The relative bioavailability of the intranasal route compared to the oral route was about 850% (916 and 793%, respectively, for animals 996 and 997).

With respect to urinary excretion, the ratio of urinary excretion of parent vs parent + metabolite was 13% by oral administration and 56% by intranasal administration, confirming that there was probably less metabolism of the drug administered by the intranasal route.

Conclusions

The cause of death on the one animal is unknown. No clinical or pathological signs other than pallor of liver and heart were found. Because of the adverse effect on food intake it was concluded that a daily dose of 12 pulses intranasal was too high and dose levels in the subsequent 13 week study were set at 1, 3 and 8 pulses based on these results.

Reviewer's Comments

1. This study was valuable mainly because it was determined that 12 pulses resulted in a marked decrease in food consumption, and was therefore too high a dose for 13 week studies.
2. The major problem with this study is that the sponsor did not include data to demonstrate the true specificity of the antibodies in the RIA's purported to measure parent drug versus drug metabolite. These results are completely dependent on the ability of these antibodies to differentiate between the two, and some verification of this would have been preferable.

TOXICOLOGY

Acute Toxicology

1. Acute toxicity study of Dihydroergotamine (DHE) 45 nasal spray solution administered by the oral or intravenous route to rats and mice. GLP, Switzerland. Sandoz Project T-203-003, 8/23/82.

The purpose of this series of studies was to determine the acute toxicity of DHE 45 Nasal Spray administered by the oral or intravenous route to rats and mice, including determination of LD_{50} if possible. These studies are summarized in the following. The product used DHE 45 Nasal Spray, 4 mg/ml.

Summary of Acute Toxicology Study Data

Species/ animals/sex/ group	Route	Dose (mg/kg)	Results	LD_{50}	Compared to prescribed human dose*
Rat; Crl: Cobs CD, 10/sex/group	Oral, gavage, single admin and observe 14 days	40 mg/kg Batch G 937	sedation (15 min post-dose), no deaths	>40 mg/kg	1300-fold higher than human
Mice; Crl:Cobs CD-1, 10/sex/group	Oral, gavage, single admin and observe 14 days	100 mg/kg Batch G 937	sedation (15 min post-dose to end observation period Day 0), no deaths	>100 mg/kg	3300-fold higher than human
Rats; Crl; Cobs CD, 10/sex/group	Intravenous, single admin and observe 14 days	20 or 40 mg/kg Batch G 937	20 mg/lkg: No deaths, ptosis and soft feces 40 mg/kg: 2/10 M, 5/10 F died 3h post-dose, muscle contractions in limbs, hypokinesia, sedation (30 sec post-dose)	Males>40 mg/kg 2/20 animals died at 40 Females=40 mg/kg	1300-fold higher than human 1300-fold higher than human
Mouse; Crl; Cobs CD, 10/sex/group	Intravenous, single admin and observe 14 days	28, 32, 26, 40, 48, 60 mg/kg Batch G 937	Mortality: Males: deaths ≥36 mg/kg Females: deaths ≥40 mg/kg at 40, death instantaneous, at < 40 death 30-60 sec post-dose Symptoms: tonic/clonic convulsions	Males=44 mg/kg Females=49 mg/kg	1466-fold higher than human 1633-fold higher than human

*prescribed human dose=2 mg/person/migraine headache=0.03 mg/kg for 60 kg person

Reviewer's Comments

These acute toxicology studies demonstrate that by the oral and intravenous routes, there is a large margin of safety (1300-3300-fold) with respect to the LD_{50} dose in animals and the prescribed human dose. Although the acute studies were not done by the intranasal route of administration, which is the route of administration proposed in the NDA, the intravenous route results in immediate introduction of a drug into plasma and often provides the lowest LD_{50} values and greatest sensitivity to toxic effects. The margin of safety for DHE 45 by the I.V. route is acceptable.

2. DHE 45 Acute toxicity study in mice, rats and rabbits, oral administration. No GLP Statement. Volume 1.5, Drug Batch 8612, Sandoz LTD, Basle, Switzerland, October, 1971.

See attached review by Kishena C. Wadhwani, Ph.D.

Subchronic Toxicology

1. Four-week intranasal toxicity study in rats. GLP. Sandoz Project #T-2849. May 21, 1992.

Study Description

Animals: Three groups of animals, each comprising 5 male and 5 female Fischer F344 rats

Treatment: DHE-45 Nasal Spray was administered 5 times daily for 28 days by the intranasal route. Dose levels were 0.4, 0.8 or 1.2 mg/day. A Control group received vehicle only. Batch No. T21018 of DHE 45 Nasal Spray (4mg/ml) in vehicle containing Dextrose and Caffeine was administered intranasal to rats by direct instillation of 10 μl droplets into each nasal orifice using a Boehringer repeat dosing pipette fitted with a tapered adaptor. Each dosing session was separated by at least 1 hour.

Observations

Mortality, clinical signs, body weight, food and water consumption, toxicokinetics, hematology, clinical chemistry, macroscopic pathology and microscopic pathology were examined.

Results

Mortality and clinical observations

There were no deaths during the study. The following clinical signs were seen almost exclusively at the high dose (1.2 mg/day) and occurred immediately after dosing: piloerection, subdued activity, foaming nostrils, breathing difficult. Subdued activity only occurred during Week 1.

Body weights and food consumption data

Males at 0.8 or 1.2 mg/day showed an inferior body weight gain of 9% and 16%, respectively, less than Controls, while females gained 6, 15 and 24% less than Controls at 0.4, 0.8 and 1.2 mg/day. Males and females showed a dose-related decrease in food consumption of 15% and 10%, respectively, less than Controls at the high dose (1.2 mg/day).

Hematology, clinical chemistry, organ weights

There were no effects on hematological parameters, clinical chemistry or organ weights (as organ/body weight ratio).

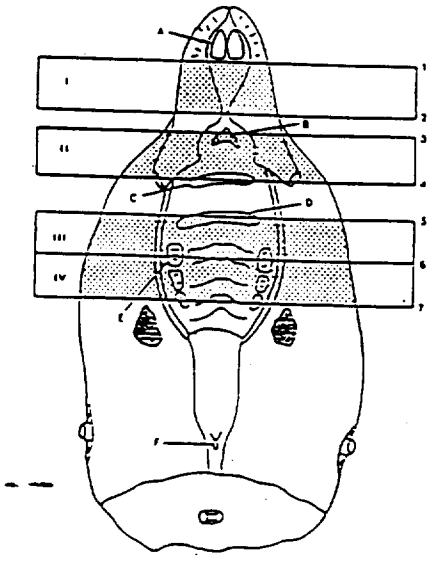
Histology

The only histology findings involved the nasal cavity. Those findings are summarized in the following Table:

	MALE				FEMALE			
NASAL CAVITY LEVEL I	0	0.4	0.8	1.2	0	0.4	0.8	1.2
Increased prominence of goblet cells								
-minimal	0	0	1	1	0	0	2	0
-slight	0	0	2	2	0	0	1	3
-Total	0	0	3	3	0	0	3	3
Focal rhinitis								
-minimal	0	2	3	1	0	2	2	1
-slight	0	4	5	5	0	0	2	3
-moderate	0	0	0	0	0	0	0	1
Luminal inflammatory exudate								
-minimal	0	0	0	0	0	0	0	1
-slight	0	0	0	2	0	0	0	2
Focal erosion								
-minimal	0	0	0	0	0	0	0	1
-slight	0	0	0	2	0	0	0	5
NASAL CAVITY LEVEL II								
Focal rhinitis								
-minimal	0	0	4	2	0	2	1	0
-slight	0	0	0	3	0	2	3	4
Increased prominence of goblet cells								
-minimal	0	0	0	0	0	0	0	1
-slight	0	0	0	0	0	0	2	0
Luminal Inflammatory exudate								
-slight	0	0	0	3	0	0	0	3
Focal erosion								
-minimal	0	0	0	1	0	0	0	2
-slight	0	0	0	1	0	0	0	2
NASAL CAVITY LEVEL III								
Luminal inflammatory exudate	0	0	0	1	0	0	0	0

Note: Nasal cavities levels I through III refer to areas of localization of symptoms that penetrate deeper into the nasal canal.
Note: A total of 5 animals per group were examined histologically.

For a diagram of the levels of the nasal cavity as per this Table, see the following diagram:



Ventral view of the rat hard palate region, with the lower jaw removed, indicating the four tissue slices. I-IV (stippled areas) which will be embedded anterior face down. The numbers on the right-hand side indicate the levels of the seven cuts necessary to produce the four slices. A upper incisor teeth; B incisive papilla; C first palatal ridge; D second palatal ridge; E first upper molar tooth; F posterior opening of the pharyngeal duct (nasopharynx).

The nasal cavity of most animals exposed to DHE 45 Nasal Spray had a minimal to slight focal rhinitis, characterized by a submucosal mixed inflammatory cell infiltrate. The distribution of incidence and severity suggested a positive relationship to dose. In animals exposed to 0.8 or 1.2 mg/day the rhinitis was in some cases accompanied by an increase in the prominence of goblet cells and at 1.2 mg by incidences of focal erosion and luminal inflammatory exudates. Most of the symptomology, with the exception of a single male, was confined to levels I and II.

Toxicokinetics and plasma levels compared to those associated with human dosing

Although plasma level measurement was mentioned in the study protocol, no data were submitted. The recommended human dose is 1 mg (1 puff in each nostril, 0.5 mg/puff) followed by a second 1 mg dose 15 minutes later for a total dose of 2 mg per headache. PK studies in humans show that this dose resulted in plasma C_{max} of about 1000-1300 pg/ml (1.1-1.3 ng/ml). This dosing regimen also resulted in AUC of about 5.7 ng.h/ml.

Perhaps the best way to compare plasma values in this subchronic toxicology study to levels resulting from prescribed clinical dosing would be to use plasma levels reported for pregnant Sprague Dawley rats in Study T-2950, a teratology study using DHE 45 Nasal Spray administered intranasal. In that study, administration of 1.2 mg/rat/day from Day 6 pc to Day 15 pc resulted in plasma C_{max} levels of 10.2 ng/ml and $AUC_{0-\infty}$ values of 56.2 ng.h/ml. 1.2 mg/day is the same dose as the highest dose administered in this subchronic toxicology study. Using these plasma values as an estimate, rats in this subchronic toxicology study probably were subjected to plasma C_{max} values about 10-fold higher and AUC values 11-fold higher than humans receiving the prescribed 2 mg DHE 45 Nasal Spray per migraine headache. The no effect level (NOEL) for "increased prominence of goblet cells" was 0.4 mg/rat/day, while there was no NOEL for "focal rhinitis".

Conclusions

The only significant toxicological effects found in this study relate to histological effects in the nasal cavity. These effects included inflammatory exudates, focal rhinitis and focal erosion limited to level II of the nasal cavity. These histological effects were consistent with the clinical symptoms exhibited by the animals. The NOEL for goblet cell effects was 0.4 mg/day, while there was no NOEL for focal rhinitis.

Reviewer's Comments

Although this degree of nasal irritation, found in a 28 day study, would be acceptable given the usefulness of the drug, with the presence of Goblet cell hyperplasia one wonders to what degree these symptoms would worsen with a more chronic administration regimen.

2. 4-week intranasal dose range finding toxicity study in mice. GLP. Sandoz Project No. T-2876, May 9, 1994.

The purpose of this study was to investigate the subchronic toxicity of DHE-45 Nasal Spray in CD-1 mice after daily intranasal administration for 28 consecutive days.

Study Description

Animals

Three groups, each comprising 5 male and 5 female CD-1 mice

Treatment

DHE-45 Nasal Spray solution administered by intranasal instillation up to 3 times daily for 28 days. Administration of the dose level was by direct instillation of 5 µl droplets into each nasal orifice using a Boehringer pipette device fitted with a tapered adaptor. Each dose session was separated by at least 2 h.

Dose

0.04, 0.08 or 0.12 mg/day of DHE 45 Nasal Spray Batch No. T21018 or vehicle solution containing dextrose (50 mg/ml) and caffeine (10 mg/ml). Untreated controls were also included in this study. Dose was chosen from previous dose-ranging study.

Dose Group	Volume (µl) Administered to Each Nostril
1. Untreated Cage Controls	0
2. Vehicle	5 µl x 1 daily
3. 0.04 mg/day	5 µl x 1 daily
4. 0.08 mg/day	5 µl x 2 daily
5. 0.12 mg/day	5 µl x 3 daily

Observations

Mortality, clinical signs, body weight, food consumption, plasma levels, hematology, clinical chemistry, organ weights and macroscopic and microscopic pathology were determined.

Hematology included: Hb, RBC, hematocrit, MCV, MCHb, WBC counts, differentials, platelet counts.

Clinical chemistry included: urea, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, total protein, chloride, albumin, A:G ratio, cholesterol, creatinine, calcium, phosphate, potassium, sodium, total bilirubin.

Histology: liver, kidney, spleen, nasal cavity (four levels), larynx, lung, trachea, cervical lymph node, bronchial lymph node, abnormal tissues.

Results

Mortality and clinical signs

No deaths were attributable to treatment with drug. Two animals died from anesthetic overdose during the clinical pathology bleed on Day 29.

There were no clinical signs associated with treatment with DHE 45 Nasal Spray. A few animals (data not presented, numbers not enumerated by sponsor) showed cyanosis sometimes accompanied by swelling on the upper side of the upper forelimbs and dorsal scapular regions in the first two weeks of the study, but this occurred in vehicle controls as well as at 0.08 and 0.12 mg/day of drug.

Bodyweight and food consumption

Male and female animals gained up to 50-60% less weight in both Vehicle Control and Treated Animals (not dose-related) than Untreated Animals, suggesting that effects on body weight could be due to either treatment (intranasal administration) per se or to the Vehicle (dextrose + caffeine) rather than to the DHE 45 (see Table below)

Group/dose (mg/day)		1♂	2♂	3♂	4♂	5♂	1♀	2♀	3♀	4♀	5♀
Body wt gain (g) weeks 0-4	Number	5	5	5	5	5	5	5	5	5	5
	Mean	7.0	2.6	4.2	2.0	2.8	3.6	2.0	3.0	1.6	2.4
	S.D.	1.7	1.1	1.3	0.7	0.8	1.5	0.7	1.4	0.5	0.5

Food consumption was decreased about 16% for males and 12% for females in both the Vehicle Control and the Treatment Groups (not dose-related), indicating that the decrease was due to either the treatment (intranasal administration) or to the contents of the Vehicle (dextrose, caffeine) and not to the DHE 45.

Toxicokinetics

Following is a Table containing plasma level values for the mice on Days 1 and 28. Samples were collected at 30 minutes post-dose in all cases.

Plasma concentrations of DHE at designated sampling time on Day 1 and Day 28 of dosing in male and female mice

Dose group (mg/day)	Male Mean plasma conc (pg/ml)	Female Mean plasma conc (pg/ml)
Day 1		
Control	BQL	BQL
Low 0.04	9456 ± 5904	14138 ± 7773
Mid 0.08	14993 ± 984	26426 ± 12759
High 0.12	27956 ± 14640	19056 ± 11249
Day 28		
Control	BQL	BQL
Low 0.04	16239 ± 18988	4261 ± 1701
Mid 0.08	13985 ± 8417	42592 ± 23818
High 0.12	22476 ± 13757	2621 ± 945

BQL=below quantifiable level

Reviewer's Comment:

Variability is very large for the plasma levels. Some evidence of a linear relationship is seen between dose and plasma levels in males on Day 1, but other than that no particular trend is seen. These values are not dramatically different on Day 28 versus Day 1, indicating that there is probably no accumulation of drug with time.

Dosing compared to prescribed human dose of 2 mg/migraine headache

The recommended human dose is 1 mg (1 puff in each nostril, 0.5 mg/puff) followed by a second 1 mg dose 15 minutes later for a total dose of 2 mg per headache. PK studies in humans show that this dose resulted in plasma C_{max} of about 1000-1300 pg/ml (1.1-1.3 ng/ml). This dosing regimen also resulted in AUC of about 5.7 ng.h/ml. Assuming the plasma levels in this mouse study to be representative of C_{max}, which may be reasonable since t_{max} in the rat was about 0.5 hour, these mice attained plasma levels of up to about 25 ng/ml drug at the high dose (0.12 mg/day). This is about 25-fold higher than the plasma C_{max} resulting from administration of the prescribed human dose.

Hematology and Clinical Chemistry

Due to the small blood samples obtained, there were insufficient clinical chemistry data to allow for a meaningful analysis. With respect to hematology, male animals showed a decrease in total WBC of about 40-50%, but this occurred both in the Vehicle Controls and the DHE 45-treated animals (not dose-related). This was reflected by a decrease of similar magnitude in lymphocytes, neutrophils and monocytes. No such decrease was seen with the female animals. These data suggest that the decrease in immune cell numbers in the male animals was not due to administration of DHE 45 but rather to either the physical trauma of intranasal drug administration or the contents of the Vehicle (dextrose, caffeine). When values for DHE 45-treated animals were compared to those for Vehicle Controls, no such decrease in immune cell numbers was apparent in animals of either sex.

Organ weights

When organ:body weight ratios were taken into account, there were no differences in organ weights (kidneys, liver, lungs, spleen) measured among the different groups. A 20% decrease in male animal liver weights was seen at the 0.08 mg/rat dose compared to Untreated Controls, but this difference disappeared with organ:body weight ratio analysis.

Necropsy and Histological Findings

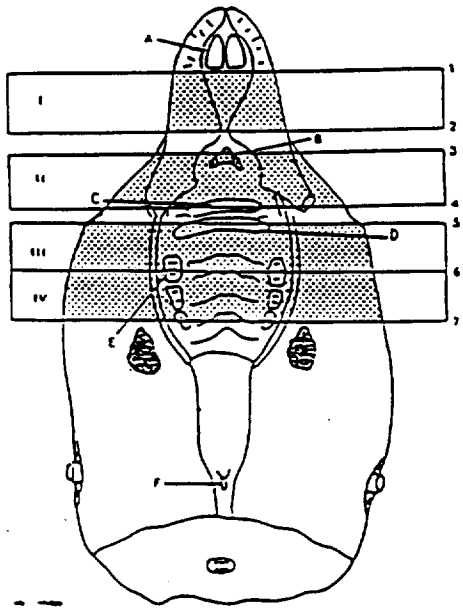
There were no abnormal necropsy findings. Histology findings related to nasal cavity pathology, with some slight effect on liver. These effects are delineated in the following Table:

DHE 45 Nasal Spray Summary of Histological Findings
Note: 5 animals per dose were examined

Organ/Parameter	Untreated Controls	Vehicle Controls	0.04	0.08	0.12
MALES					
Liver					
-Focus of inflammation					
-minimal	-	0	1	0	0
-slight	-	0	1	0	0
-Total	-	0	2	0	0
Nasal Cavity Level I					
Rhinitis					
-minimal	-	1	2	2	2
-slight	-	0	0	1	3
-Total	-	1	2	3	5
Eosinophilic inclusions in respiratory epithelium					
-minimal	-	0	1	3	1
-slight	-	0	3	1	2
Total	-	0	4	4	3
Eosinophilic inclusions in olfactory epithelium					
-minimal	-	0	0	0	1
-slight	-	0	0	1	0
Total	-	0	0	1	1
Goblet Cell Hyperplasia					
-minimal	-	0	0	0	2
Nasal Cavity Level II					
Rhinitis					
-minimal	-	0	0	2	2
-slight	-	0	0	1	2
Total	-	0	0	3	4
Eosinophilic inclusions in respiratory epithelium					
-minimal	-	0	3	1	2
-slight	-	0	0	2	2
Total	-	0	3	3	4

FEMALES					
Liver					
Focus of inflammation					
-minimal	-	0	2	1	3
-slight	-	2	0	0	0
Total	-	2	2	1	3
Nasal Cavity Level I					
Rhinitis					
-minimal	-	0	3	1	1
-slight	-	0	1	3	4
Total	-	0	4	4	5
Eosinophilic inclusions in respiratory epithelium					
-minimal	-	1	2	1	1
-slight	-	0	2	3	3
-moderate	-	0	0	0	1
Total	-	1	4	4	5
Goblet Cell Hyperplasia					
-minimal	-	0	1	0	2
-slight	-	0	0	1	0
Total	-	0	1	1	2
Nasal Cavity Level II					
Rhinitis					
-minimal	-	2	2	4	0
-slight	-	0	3	1	3
Total	-	2	5	5	3
Eosinophilic inclusions in respiratory epithelium					
-minimal	-	0	1	1	0
-slight	-	1	4	4	3
-moderate	-	0	0	0	1
Total	-	1	5	5	4

For a diagram of the various levels of the nasal cavity of the mouse, see the following:



Ventral view of the mouse hard palate region, with the lower jaw removed, indicating the four tissue slices. I-IV (stippled areas) which will be embedded anterior face down. The numbers on the right-hand side indicate the levels of the seven cuts necessary to produce the four slices. A upper incisor teeth; B incisive papilla; C first palatal ridge; D second palatal ridge; E first upper molar tooth; F posterior opening of the pharyngeal duct (nasopharynx).

Histology findings were similar to those reported for the rat and involved mainly symptoms of irritation in the nasal cavity, such as rhinitis and presence of eosinophilic inclusions in respiratory and olfactory epithelium. These symptoms were limited to the depth in the nasal cavity of Levels I and II. However, **of greater concern was the finding of goblet cell hyperplasia in nasal cavity level I.** This condition was reported in 2 high-dose male mice and 1 low-dose and 2 high-dose female mice, and constitutes a somewhat more severe response to irritation of the intranasal administration of DHE 45. Hyperplasia is a non-neoplastic form of cell proliferation, generally referring to an absolute increase in numbers of cells in a tissue or organ. This condition can arise as the result of chronic inflammation. Although generally regarded as non-neoplastic, neoplasms sometimes do arise out of these areas of proliferation (*Principles of Toxicological Pathology*, Glaister, J.R., Taylor & Francis Publishers, London and Philadelphia, 1986). Also of importance is the fact that there was no NOEL for goblet cell hyperplasia in female animals, nor was there a NOEL apparent for a number of the other histopathological effects in the nasal cavity in this study.

It is unclear whether or not the inflammatory foci in livers of males (2/5 high dose) and females (3/5 high dose) are significant biologically. There was some evidence of lower liver weights in males, but this disappeared when data were analyzed as organ:body weight ratio.

Conclusions

The only effects of note in this study were those locally occurring in the nasal cavity, including rhinitis, eosinophilic inclusions and goblet cell hyperplasia. These are symptoms indicative of chronic inflammatory response, especially in the case of the hyperplasia. The sponsor chooses to conclude that "intranasal application of DHE 45 Nasal Spray to CD-1 mice at dose levels of 0.04, 0.08 and 0.12 mg/day for 28 consecutive days produced a minimal to slight focal rhinitis and eosinophilic inclusions in the nasal respiratory epithelium." The sponsor did not address the goblet cell hyperplasia. These effects occurred in a 28 day study, and their greatest significance is in raising the level of concern as to what might happen with much longer exposure. These data, especially the presence of goblet cell hyperplasia, suggest that the results of the carcinogenicity studies by intranasal administration will be very important in evaluating the safety of DHE 45 Nasal Spray. The lack of a NOEL for a number of these effects, including goblet cell hyperplasia in females, further emphasizes this point.

Reviewer's Comments

The most important findings in this study are the histopathological findings, including the non-neoplastic proliferative condition of goblet cell hyperplasia, in the nasal cavity levels I and II which are indicative of the presence of chronic inflammation. The fact that this condition is present after 28 days of intranasal administration of DHE 45 raises the question in my mind as to the possibility of an even worse scenario with longer exposure times, maybe even including neoplasia. Therefore, the carcinogenicity studies done by intranasal administration of DHE 45 may be very important in determining the level of safety of this drug.

3. 13-week intra-nasal toxicity study in the Cynomolgus monkey. No GLP statement. Sandoz [redacted], May 1984.

The purpose of this study was to evaluate the toxicity of DHE 45 spray, administered intranasal to the Cynomolgus monkey for 13 weeks.

Study Description

Test Article

DHE 45 Nasal Spray Batch #G005, 4 mg/ml.

Animals

24 wild caught Cynomolgus monkeys (Macaca fascicularis) from [redacted]
3/sex/group.

Route of Administration

Intranasal administration using modified human applicators with animals held horizontally, face upwards during dosing.

Dose levels, frequency and duration of administration

These doses were selected for the study based on examination of data from a 17 day range-finding study.

Group (mg/animal/day)	Dosage Total number of pulses daily	Regimen
1 Control (0)	8 (vehicle)	2 pulses per nostril twice daily
2 Low (0.46)	1	1 pulse (left nostril) daily
3 Intermediate (1.38)	3	1 pulse per nostril (A.M.) 1 pulse (left nostril) (P.M.)
4 High (3.68)	8	2 pulses per nostril twice daily

Observations

Clinical signs and behavior, body weights, food consumption, hematology (Hb, MCV, RBC, MCH, PCV, MCHC, WBC, differentials, prothrombin time and APTT), clinical chemistry (GOT, GPT, Alk.P, sodium, potassium, chloride, glucose, BUN, total protein, albumin), urine analysis, organ weights, macroscopic and microscopic pathology were evaluated. Nasal tissue samples were taken from each animal for electron microscopy analysis.

Results

Mortality

One female animal (Group 3; 1mg/animal/day) developed diarrhea on Day 15, was tested for bacterial and parasitic infections and found to be negative for these, was found moribund of Day 18 and was sacrificed. Blood samples revealed only changes consistent with dehydration. No macroscopic findings or organ weight abnormalities were found, and cause of death was stated as intractable diarrhea, possible of viral origin. There were no other animal deaths.

Clinical signs

The only treatment-related clinical symptom was epistaxis (bleeding from the nose), which occurred in a number of instances as delineated below:

Individual treatment-related clinical observations

Group and sex	Animal numbers	Week of Observation	Clinical Observation
1M	51-53		No abnormalities detected.
1F	63	7	Cream discharge from left nostril.
	64-65		No abnormalities detected.
2M	54-56		No abnormalities detected.
2F	66		No abnormalities detected.
	67	4	Dried blood inside left nostril
	68	6	Slight bleeding from left nostril about 5 minutes after dosing.
3M	57		No abnormalities detected.
	58	4	Bleeding from both nostrils immediately after dosing.
	59	2-3	Dried blood inside and outside both nostrils; inflammation and part or full closure of left eye.
		4	Dried blood inside both nostrils.
3F	69-71		No abnormalities detected.
4M	60		No abnormalities detected.
	61	4	Dried blood inside both nostrils; peri-orbital swelling of both eyelids, inflammation and part or full closure of right/both eyes accompanied by purulent discharge, decreased severity following bathing with saline solution.
	62		No abnormalities detected.
4F	72		No abnormalities detected.
	73-74	10	Bleeding from nose.
			No abnormalities detected.

Reviewer's Comment:

These data would seem to indicate that the bleeding from the nose is due to an effect of the drug and not to the physical trauma of intranasal administration, since no bleeding was reported in the Vehicle Control animals. This level of irritation due to drug alone would suggest that human administration may also be associated with some degree of discomfort.

Body weights

Although the sponsor concluded that there was no effect of the drug on body weight, there was actually a slight decrease (about 9%) in body weight of the female animals in the High Dose (mean body wt. for all 13 weeks = 2.49±0.0 kg) compared to Vehicle Controls (2.74±0.06 kg mean body wt. for all 13 weeks). This may suggest that female animals were more susceptible to toxicity from the drug than males.

Food consumption

There was not effect of the drug on food consumption.

Ophthalmoscopy

There were no treatment-related ocular changes.

Nasal examinations

Animals receiving DHE 45 intranasal at the various doses presented with mucosal ulceration with scabbing or with hemorrhage. On Week 4, 13 of 18 treated animals (Group 1, 0/6; Group 2, 5/6; Group 3, 3/5 and Group 4, 4/6) presented with ulceration, Week 8, 9 of 18 presented with ulceration, and on Week 13, 4 of 18 animals presented with ulceration. Therefore, the incidence of nasal mucosal

ulceration, though high at the beginning of the study, seemed to resolve itself by the end of the 13 weeks. Other symptoms in the nasal cavity included nasal bleeding and mucopurulent discharge.

Electrocardiograms
There were no abnormalities associated with heart rate or conduction with DHE 45.

Hematology
There were no effects on hematological parameters.

Clinical chemistry and urinalysis
There were no effects on clinical chemistry or urinalysis parameters.

Organ Weights
There were no effects on organ weights when analyzed as organ:body weight ratio.

Histopathology
The only histopathology findings were related to the nasal cavity and the lung. With respect to the nasal cavity, minimal rhinitis (1 LDM, 1HDM) and congestion of submucosal blood vessels (1LDF) were reported. With respect to the lung, congestion (1 MDF), pigmented histiocytes (1 Control M, 1 LDM, 1 MDM, 1 HDM, 1 LDF, and 2 HDF), and pneumonitis (1 MDM, 1 Control F, 1 LDF, 1 MDF) were seen. Three animals per group were examined in these studies.

Reviewer's Comment
The sponsor states that these effects were considered not to be definite treatment-related local effects. However, it is my estimation, based on the clinical signs and symptoms and the results of previous animal studies with DHE 45, that at least the nasal cavity histopathology results are treatment-related.

Toxicokinetics
Blood samples were taken from all animals during Weeks 5 and 12, and results were reported elsewhere. The following Table contains those toxicokinetics data.

Toxicokinetic data from 13-week intranasal toxicology study in Cynomolgus monkeys-minimum plasma concentrations

Group (mg/animal/day)	Week 5		Week 12	
	Parent drug* (ng/ml)	Parent drug + metabolite	Parent drug (ng/ml)	Parent drug + metabolite
1. Vehicle (0)	–	–	–	–
2. Low dose (0.5 mg/day)	0.128	0.187±0.161	–	0.273
3. Intermediate dose (1 mg/day)	0.153±0.099	0.389±0.121	0.127±0.079	0.186±0.174
4. High dose (4 mg/day)	1.050±0.712	1.693±1.267	0.616±0.372	0.680±0.523

*Data are means of plasma values for 6 animals per group.

These data demonstrate that the drug was absorbed when administered by the intranasal route. Absorption was variable. With this degree of variability , it is difficult to determine whether or not plasma level increases linearly with dose. However, at Week 5, plasma level does increase with dose, and the relationship appears to be somewhat linear. Plasma levels are somewhat lower with drug administration at Week 12 than at Week 5. Since these are trough levels rather than plasma C_{max} levels, this could reflect an increased metabolism of the drug, or it could be due to a saturation of absorption.

The mean ratio of the parent drug over the parent drug + metabolite was 64% for groups 3 and 4, a value which is in agreement with the results of the preliminary study in which on the basis of the AUC this proportion was 71%.

Dosing compared to prescribed human dose

The dosing regimen was designed to mimic the prescribed human dosing regimen and the same DHE 45 Nasal Spray apparatus was used that is used in the clinic. The recommended human dose is 1 mg (1 puff in each nostril, 0.5 mg/puff) followed by a second 1 mg dose 15 minutes later for a total dose of 2 mg per headache. PK studies in humans show that this dose resulted in **plasma C_{max} of about 1000-1300 pg/ml (1.1-1.3 ng/ml)**. This dosing regimen also resulted in AUC of about 5.7 ng.h/ml.

There is a problem with comparing plasma levels between the those derived from the prescribed human dosing regimen for a migraine headache and those in the monkeys in this study, because the monkey plasma levels represent trough levels while the human plasma levels represent C_{max}. From the fact that the human plasma C_{max} levels were 1.1-1.3 ng/ml and the monkey trough levels at the high dose (4mg/animal/day) were about 1 ng/ml, it is safe to say that the Cynomolgus monkeys were certainly exposed to higher levels than the humans receiving the prescribed dose of 2 mg/migraine headache. However, it is difficult to determine the degree of difference in the two.

Another way to compare the dosing in the Cynomolgus monkey study with the prescribed human dose is to analyze the dose by body surface area. The Cynomolgus monkeys received a maximum of 4 mg/animal/day. Those animals weighed an average of about 2.5 kg/animal. 4 mg per 2.5 kg would be about 1.6 mg/kg/day. Analyzed in terms of surface area, this is equivalent to a human dose of about 0.53 mg/kg/day (multiply monkey dose x 1/3 to convert). The human dose is 2 mg per headache, which for a 60 mg person is about 0.03 mg/kg. Therefore, by body surface area analysis, the Cynomolgus monkeys in this study received about **18-fold more drug at the high dose than a human** would receive with the prescribed human dose. This analysis is probably valid because the bioavailability of DHE 45 by the intranasal route compared to the intravenous route is about 40% for both humans and Cynomolgus monkeys.

Conclusions

Clinical symptoms data indicate that administration of DHE 45 Nasal Spray by the intranasal route is probably quite irritating to the nasal cavity, at least early in the study. This is witnessed by the prevalence of nasal bleeding and ulceration. However, over the 13 week study the incidence of these symptoms appears to decrease considerably. The lack of blatant histopathological changes in the nasal cavity of the animals to accompany these clinical symptoms could indicate that the animals were able to adapt to the nasal irritation.

There is some evidence that the incidence of pneumonitis increased in the treated animals. However, these data are more difficult to interpret because one female control animals also demonstrated this pathology and the increased pneumonitis did not appear to be entirely dose-related.

Reviewer's Comments

The minimal histopathology findings in the nasal cavity of the Cynomolgus monkeys was somewhat surprising in light of the finding in the four-week rat and mouse studies. It is possible that if the rat or mouse studies were carried out to 13 weeks, the animals may have also adapted to the administration of the drug. However, there were still some histopathological findings in the nasal cavities of all three species of animals from all three of these studies, and these results suggest that the results of the rodent carcinogenicity studies by the intranasal route will be very important in assessing the overall safety of this drug. Furthermore, the decrease in incidence of Cynomolgus monkey nasal bleeding over the 13 weeks could possibly be explained by the vasoconstrictive properties of the drug and of the caffeine in the preparation. Such vasoconstrictive activity might decrease the amount of blood perfusing the nasal cavity, and reduce bleeding in this manner.

4. Dihydroergotamine mesylate (DHE-45) A 26-week oral toxicity study in rats (Project #201-001). No GLP Statement. Volume 1.5, Batch #8612, Sandoz LTD., Basel, Switzerland, December, 1969.

See attached review by Kishena C. Wadhwani, Ph.D.

5. Dihydroergotamine (DHE-45), A 26-week oral toxicity study in dogs (Project #201-002). No GLP Statement. Volume 1.5, Batch #8612, Sandoz LTD, Basel, Switzerland, December, 1969.

See attached review by Kishena C. Wadhwani, Ph.D.

6. DHE 45 Nasal Spray 3 month intranasal maximum tolerated dose study in rats (Project #T-2898). GLP. Report No. 11104, June, 1994.

The purpose of this study was to investigate the toxicity of DHE 45 Nasal Spray in Fischer 344 rats after daily intranasal administrations for 13 weeks. The study was also intended to demonstrate locally induced toxicity in the respiratory tract and/or systemic toxicity following administration of DHE 45 by the intranasal route.

Study Description

Test Article
DHE 45 Nasal Spray (4 mg/ml), Batch No. T-21018.

Animals
One hundred and sixty-eight (84 male, 84 female) Fischer 344 rats, 5-5 weeks old, males 89-113 grams, females 94-111 grams.

Treatment
Each group consisted of 10/sex/group Main Study animals and a Satellite group of animals (4/sex/group) for toxicokinetics determinations. Study groups were as follows:

Dose Group	Dose (mg DHE-45/animal/day)	Volume Administered (µl/nostril)
1	0 (vehicle only)	30 x 5 daily
2	0.08	5 x 2 daily
3	0.32/1.6*	20 x 2 daily
4	0.72	30 x 3 daily
5	1.2	30 x 5 daily

*From the beginning of Week 8 (i.e. Day 50) the dose level for the Group 3 animals was increased from 20 µl/nostril x 2 daily to 40 µl/nostril x 5 daily. This change in treatment regimen was agreed with the Sponsor and was considered necessary due to the lack of any treatment related effect. In each case, both nostrils were included at each dosing.

Observations
Study observations were as follows: clinical symptoms, body weight, food consumption, water consumption, ophthalmoscopy, hematology (Hb, RBC count, hematocrit, MCV, MCHb, WBC count, differentials, platelets, reticulocytes), clinical chemistry (blood urea nitrogen, glucose, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, total protein, sodium, potassium, chloride, albumin, globulin, A:G ratio, cholesterol, triglycerides, creatinine, calcium, phosphate, total

bilirubin, protein electrophoresis), urinalysis, organ weights (adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, pituitary, spleen, testes, thymus, thyroids and uterus), macroscopic and microscopic pathology, nasal cavity pathology. A full histological examination of Control (Group 1) and High dose (Group 3, 1.6 mg/animal/day) animals for a full range of tissues was carried out. In addition, the respiratory tract tissues were examined from the Low and Intermediate dose groups (Groups 2, 4 and 5). Tissues included abnormal tissues, adrenals, aortic arch, brain, clitoral gland, femur, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, heart, kidneys, liver, lung, mammary gland, mediastinal lymph node, mesenteric lymph node, esophagus, pancreas, pituitary, preputial gland, prostate, sciatic nerve, skin, seminal vesicles, spinal cord, spleen, sternum/rib, submandibular lymph node, submaxillary salivary gland, thigh muscle, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, nasal cavity, larynx, cervical lymph node ovaries/testes, bronchial lymph node. Femoral bone marrow smears were also done.

Results

Mortality

Two animals died during the course of the study. One female (Group 2, 0.08 mg/animal/day) was sacrificed for humane reasons at the end of Week 13, due to badly bulging eyes following retro-orbital plexus blood sampling. One female animal (Satellite Group 5, 1.2 mg/animal/day) was found dead on Day 80. No cause of death was given, but the sponsor stated that this death was not considered treatment related.

Clinical signs

The sponsor stated that "there were no obvious signs related to treatment. Several of the animals showed staining and encrustations around the nose and eyes. All clinical observations were considered to be incidental to treatment." Following is a numerical summary of these clinical observations:

Summary of incidences of clinical reportings of "red staining of nose" and "nose red/encrusted":

Group	Incidence of observations
1	19
2	11
3 (1.6 mg/day)	19
4	20
5	17

Reviewer's Comment

Since 19 occurrences of this symptomology were reported in the Vehicle Control, these data suggest that in this study that some of the irritation of the nasal cavity was due to the physical treatment of the animals rather than to the DHE 45 itself.

Body weight

The only effect of drug administration on body weight was seen in the Group 3 animals that were turned into the High Dose Group by modifying the protocol to administer 1.6 mg/animal/day based on the lack of toxicity in the original High Dose Group (Group 5) receiving 1.2 mg/animal/day. These animals demonstrated a slight overall weight loss (-5 g) over Weeks 8-13.

Food Consumption

Group 3 animals (ultimately receiving 1.6 mg/animal/day) showed a reduction in food consumption (about 11% for males, 1% for females) during Weeks 8-13.

Ophthalmic Evaluations
No treatment-related effects.

Hematology
Some fluctuation of immune cell numbers did occur. With respect to total white blood cells (WBC), WBC number was decreased with increasing doses of DHE 45 in males (7%, 1.2 mg/animal/day) and females (18%, 1.2 mg/animal/day). Of greater interest was the fact that primarily the neutrophils were effected, with a dose-related decrease in both males (0.08 mg/day, 0%; 0.72 mg/day, 4%; 1.2 mg/day, 20%) and females (0.08 mg/day, 17.5%; 0.72 mg/day, 27%; 1.2 mg/day, 50%). The neutrophile data were statistically significant in the females at a level of $p<0.05$. The results were not due to one or two outlying values but rather to a decrease in neutrophile numbers from a majority of the animals in each group. The data for Group 3, the group that began as 0.32 mg/day and was converted to a High Dose of 1.6 mg/day, demonstrated values for WBC and neutrophils higher than the 1.2 mg/day group, which is not surprising since these animals did not receive the 1.6 mg/day dose for the entire 3 months.

Reviewer's Comments

These data suggest that DHE 45 may preferentially effect neutrophils. A decrease in neutrophile numbers of up to 50% at 1.2 mg/animal would certainly fit into the class of a biologically significant effect. These neutrophile data are particularly interesting in light of the fact that the total WBC count is only decreased about 18% a the 1.2 mg/animal dose. The effect appears to be greater in female than male animals.

Clinical Chemistry
Although a number of clinical chemistry parameters fluctuated somewhat, the only alteration consistent between the sexes and of a dose-related nature was an increase in LDH. Both males (0.08 mg/day, 10%; 0.72 mg/day, 27%) and females (0.08 mg/day, 1.3%; 0.72 mg/day, 5.5%; 1.2 mg/day, 16.8%) demonstrated a dose-related increase in LDH. However, these increases would have to be classified as small.

Urinalysis Data
No effects were seen.

Organ Weights
Although fluctuations occurred, not apparently dose-related effects were seen.

Macroscopic Pathology
The only macroscopic pathological findings of note were with respect to the heart and the nasal cavity and are delineated in the following table with respect to Level of Occurrence in the Nasal Cavity (Levels 1-4). For the diagram of the levels of the rat nasal cavity, see page 10 of this review.

Findings	Incidence of Lesions (numeric)									
	Males					Females				
	Grp 1	Grp2	Grp4	Grp5	Grp3	Grp 1	Grp2	Grp4	Grp5	Grp3
	0 mg	0.08	0.72	1.2	1.6	0 mg	0.08	0.72	1.2	1.6
HEART:										
Focal myocarditis	5				7	1				3
NASAL CAVITY:										
Focal respiratory epithelial hyperplasia										
LEVEL I:										
Very mild	2	1	1	5	3	1	2	1	5	1
Mild	0	0	0	0	3	0	0	6	2	5
Moderate	0	0	0	0	0	0	0	0	0	4
LEVEL II:										
Very mild	0	0	0	0	0	0	0	1	0	0
Mild	0	0	0	0	0	0	0	1	1	1
Goblet-cell proliferation										
LEVEL I:										
Very mild	0	1	4	5	7	0	0	2	8	7
Mild	0	0	3	4	3	0	2	8	1	7
LEVEL II:										
Very mild	0	0	2	1	5	0	0	2	4	1
Mild	0	0	2	4	3	0	0	7	1	5
Respiratory epithelial eosinophilic inclusions										
LEVEL I:										
Very mild	0	0	1	0	0	0	0	0	0	0
LEVEL II:										
Very mild	0	0	2	2	0	0	0	3	1	2
Mild	0	0	0	0	0	0	0	0	6	1
Olfactory epithelial eosinophilic inclusions										
LEVEL II:										
Very mild	0	0	0	1	0	0	0	4	0	2
Mild	0	0	0	0	0	0	0	2	0	2
LEVEL III:										
Very mild	0	0	1	1	0	0	0	3	5	3
LEVEL IV:										
Very mild	0	0	0	0	0	0	0	0	2	1
Mild	0	0	0	0	0	0	0	2	4	0
Focal mucosal inflammatory cell infiltrate										
LEVEL I:										
Very mild	5	3	1	0	0	1	5	0	0	0
Mild	0	0	0	0	0	3	0	0	0	0
LEVEL II:										
Very mild	4	4	3	0	0	3	7	0	0	0
Mild	0	0	0	0	0	3	0	0	0	0
Intraluminal inflammatory exudate										
LEVEL I:										
Very mild	0	0	0	0	0	0	0	3	0	1
Mild	0	0	0	0	0	0	0	0	0	3
LEVEL II:										
Very mild	0	0	0	0	1	0	0	0	0	0
Mild	0	0	0	0	0	0	0	0	0	2

Diffuse rhinitis										
LEVEL I:										
Very mild	0	3	5	7	2	0	0	2	1	0
Mild	0	0	2	3	7	1	1	8	8	3
Moderate	0	0	0	0	1	0	1	0	1	7
LEVEL II:										
Very mild	0	0	1	7	2	0	0	2	0	0
Mild	0	0	2	3	6	0	0	8	8	3
Moderate	0	0	0	0	1	0	0	0	1	7
Focal squamous metaplasia										
LEVEL I:										
Very mild	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**2**	**0**	**2**
Focal cellulitis										
LEVEL I:										
Mild	0	0	0	0	0	0	0	0	1	0
Intraluminal mucous										
LEVEL I:	0	0	0	0	1	0	0	0	1	3
LEVEL II:	0	0	0	0	0	0	0	0	1	1

In the nasal cavity, a very mild to moderate, diffuse, bilateral rhinitis, consisting of a submucosal mixed inflammatory cell infiltrate, was present in the anterior nasal cavity of all animals receiving 1.2 or 1.6 mg DHE 45/day, all females and 7 males receiving 0.72 mg/day and one female and 3 males receiving 0.08 mg/day. Most animals with rhinitis had an associated very mild or mild goblet cell proliferation. Focal hyperplasia of the respiratory epithelium was present in most females with rhinitis but in males it was present in 6 receiving 1.6 mg/day and 5 receiving 1.2 mg/day. Other findings included eosinophilic cell inclusions, inflammatory cell infiltrates and exudates, and squamous metaplasia, occurring in females only.

For the majority of symptoms in the nasal cavity, there were no definitive no effect levels (NOEL) found. With respiratory epithelial eosinophilic inclusions, olfactory epithelial eosinophilic inclusions and intraluminal inflammatory exudate, a NOEL of 0.08 mg/kg is apparent. However, with the hyperplasias and squamous metaplasia in females, no NOEL was found.

The findings of main concern in this table are the high incidence of Goblet-cell hyperplasia (proliferation), focal respiratory epithelial hyperplasia and the low level of squamous metaplasia. The Goblet-cell hyperplasias were usually found in animals that demonstrated rhinitis. Hyperplasia and metaplasia are pathological findings that are usually indicative of chronic irritation. Although categorized as non-neoplastic proliferative conditions, squamous metaplasia, under conditions of persistent chronic injury, may become disorderly with variations in cell size, orientation and staining properties. This is termed atypical squamous metaplasia, and in the bronchial epithelium of cigarette smokers this is a frequent precursor of bronchial neoplasms. A similar situation exists for the hyperplasias.

Reviewer's Comment

These findings are consistent with the other intranasal subchronic toxicology studies in Cynomolgus monkey, rat and mouse in that the main effects involved clinical symptoms and pathological effects related to the nasal cavity.

Concrete conclusions regarding the histopathological data in the nasal cavity of these animals are difficult to formulate, since the metaplasias and hyperplasias can either be a simple sign of chronic inflammation/irritation without ever giving rise to neoplasia, or in some instances neoplasias can arise out of these non-neoplastic proliferative conditions. The fact that these effects are mostly absent from control animals indicates that these effects are due to the drug itself and not to physical trauma of intranasal administration. **These results point to the carcinogenicity studies in rodents by the intranasal route as being very important in analyzing the safety of DHE 45.**

Toxicokinetics

The following table shows the dosing schedule for this experiment again, to allow interpretation of the toxicokinetics data.

Dose Group	Dose (mg DHE-45/animal/day)	Volume Administered (µl/nostril)
1	0 (vehicle only)	30 x 5 daily
2	0.08	5 x 2 daily
3	0.32/1.6*	20 x 2 daily
4	0.72	30 x 3 daily
5	1.2	30 x 5 daily

*From the beginning of Week 8 (i.e. Day 50) the dose level for the Group 3 animals was increased from 20 µl/nostril x 2 daily to 40 µl/nostril x 5 daily. This change in treatment regimen was agreed with the Sponsor and was considered necessary due to the lack of any treatment related effect. In each case, both nostrils were included at each dosing.

Table 1 below shows the toxicokinetics data for this study.

Table 1 plasma concentrations of DHE in study T-2898

Dose (mg/day of DHE)		WEEK 1 MALE	WEEK 1 FEMALE	WEEK 12 MALE	WEEK 12 FEMALE
0	Cmax*	BQL***	BQL	8949	6800
	AUC**	0	0	44800	32400
0.08	Cmax*	693	1465	754	1780
	AUC**	12200	1470	2060	1780
0.32	Cmax*	4414	6003	-	-
	AUC**	36800	33700	-	-
0.72	Cmax*	10978	10026	17752	17524
	AUC**	79600	83200	98500	66100
1.2	Cmax*	14362	16976	21036	11149
	AUC**	117000	111000	149000	120000
1.6	Cmax*	-	-	18670	16642
	AUC**	-	-	198000	163000

Note: samples were taken at 0.5, 1, 4 and 20 hours after administration of the last dose. Tmax occurred almost exclusively at 0.5 hour. *Cmax=pg/ml **AUC=AUC_{0-} ***BQL=below quantifiable level.

There was a problem with the Control animals at the 12 week time point. At 1 Week, there was no DHE 45 detected in Control animals, whereas, at 12 Weeks a fairly large amount of the drug was detected. The sponsor stated that this was due to accidental contamination during sample handling or collection.

These data demonstrate that DHE 45 was absorbed fairly rapidly by the intranasal route of administration. There appear to be no time-dependent pharmacokinetics by this route. However, there does appear to be a plateau of plasma levels with dose that occurs between 0.72 and 1.2 mg/day of DHE 45. This occurs to some extent on both Week 1 and Week 12 and with both sexes. These data could be interpreted to indicate a saturation of absorption of the drug at the higher doses. Furthermore, there appears to be no difference with respect to gender of the animals during Week 1. However, in Week 12 the AUCs appeared to be somewhat greater in males than females.

Plasma concentrations with respect to human prescribed dose

The following is a table comparing rat exposure to DHE 45 in this 3-month study to the prescribed human dose of 2 mg per migraine headache by three different methods, plasma C_{max} and AUC, total dose, and dose per surface area of nasal cavity in mg per mm^2.

Species	Dose (mg)	Comparison to human per PLASMA LEVELS (ng/ml) AUCS (ng.h/ml)	at prescribed human migraine TOTAL DOSE	dose of 2 mg headache DOSE PER SURFACE AREA OF NASAL CAVITY (MG/MM^2)
Rat	low dose (0.08 mg)	plasma C_{max} rat=1.1 human=1-1.3 HUMAN AND RAT IN SAME RANGE AUCs rat=1.5-2.0 human=5.7 HUMAN 2-FOLD>RAT	Rat=0.08 mg Human=2.0 mg HUMAN 25-FOLD>RAT	Rat=0.00006 Human=0.0001 HUMAN AND RAT IN SAME RANGE
Rat	high dose (1.6 mg)	plasma C_{max} rat=20 human=1-1.3 RAT 20-FOLD>HUMAN AUCs rat=150-200 human=5.7 RAT 26-35-FOLD>HUMAN	Rat=1.6 mg Human=2.0 mg RAT AND HUMAN IN SAME RANGE.	Rat=0.0012 Human=0.0001 RAT 12-FOLD>HUMAN

There are at least three ways to compare the drug exposure of the animals in this study to the drug exposure for a human receiving the prescribed clinical dose of 2.0 mg per migraine headache, as seen in the above Table. The following discussion is organized according to those different methods.

Plasma C_{max}/AUC

The best way to compare DHE 45 exposures in animals in this 3-month study versus the human receiving the prescribed clinical dose for a migraine headache, when one considers systemic toxic effects of the drug, is probably to compare plasma levels and AUCs. The recommended human dose is 1 mg (1 puff in each nostril, 0.5 mg/puff) followed by a second 1 mg dose 15 minutes later for a total dose of 2 mg per headache. PK studies in humans show that this dose resulted in **plasma C_{max} of about 1000-1300 pg/ml (1.1-1.3 ng/ml)**. This dosing regimen also resulted in AUC of about 5.7 ng.h/ml.

Rats receiving the lowest dose, 0.08 mg/day, when compared to humans with respect to plasma levels and AUC, demonstrated about the same plasma levels as humans receiving the prescribed dose. With respect to AUCs, the human received about 2-fold higher exposure to the drug than the rat. With respect to the highest dose, 1.6 mg., rats in this 3 month study were exposed to plasma C_{max} levels of up to about 20,000 pg/ml (20 ng/ml) and $AUC_{0-\infty}$ of up to 150-200,000 pg.h/ml (150-200 ng.h/ml). This is about 20-fold greater plasma levels and about 26-35-fold greater AUC than attained in humans with the prescribed dose for a migraine headache.