Total Dose

With respect to the drug side effect found in the nasal cavity, systemic exposure is probably not as relevant as local exposure to the drug in the area of the nasal cavity. One way to compare exposure levels between rat and human is to simply compare the total dose administered to the nasal cavity in the two species.

With respect to the lowest dose, 0.08 mg/day, administered to the rats, the human receiving the prescribed clinical dose of 2 mg per migraine headache would receive about a 25-fold greater exposure in terms of total dose than the rats at this dose. Considering the highest dose administered to the rats, 1.6 mg/day, the rat and the human would be exposed to approximately the same level of drug in the nasal cavity in terms of total dose.

Dose Per Surface Area of Nasal Cavity (mg/mm^2)

Another, perhaps more accurate, method of comparing rat and human exposure to DHE 45 in the nasal cavity is to compare the two in terms of drug dose (mg) per nasal cavity surface area (mm^2). This method allows for normalization of the total dose of drug in terms of local surface area to which the drug is being administered. In these terms at the rat low dose the human and the rat would be receiving about the same level of drug exposure. At the rat high dose, the rat would be exposed to a drug level about 12-fold greater than the human.

Reviewer's Comments regarding exposure comparisons

It is very important to note that this study did not reveal a NOEL for the majority of the nasal cavity symptoms, including the Goblet cell hyperplasia, focal respiratory epithelial hyperplasia, and squamous metaplasia (females). These symptoms occurred even at the low dose of 0.08 mg/day. If one uses the plasma levels/AUC method or the dose per surface area method for comparison, this means that these nasal cavity effects were occurring at a drug exposure level very similar to that expected for the human receiving the prescribed clinical dose. If one uses the total dose method of comparison, then one would interpret these data to mean that these nasal cavity effects are occurring in rat at plasma drug exposure levels 25-fold less than the human would be expected to experience. No matter which way one looks at these data, there is absolutely no margin of safety for this drug with respect to these nasal cavity effects. And in fact the situation is even worse in that, due to the lack of a NOEL for these effects, it is not really known at what drug exposure levels these histopathological symptoms might cease to occur. Therefore, the data appear even worse in this light. These data could certainly be interpreted to predict that patients receiving the prescribed dose of drug for a migraine headache would most likely experience similar nasal cavity symptoms to those found in the rat in this study, or at least to experience some form of chronic irritation/inflammation.

Overall conclusions

Results of this study again point to nasal cavity effects. Of major importance is the fact that in this 3-month study there appears to be a progression of symptoms from the 4 week study in rats to a higher incidence of Goblet cell hyperplasias and the appearance of respiratory epithelial cell hyperplasia and squamous metaplasia that were not apparent in the 4-week study. Therefore, results of this 3-month study in rats indicate that the chronic inflammatory effects of the drug on the nasal cavity can result in a progressively worse set of histopathological findings. The fact that any such symptoms are mostly absent from Vehicle Control animals indicates that this chronic inflammatory effect is, in fact, due to the drug itself. This study further raises the question of progression of symptoms with time, and further emphasizes the importance of the carcinogenicity studies in determining the safety of this drug by the intranasal route.

Furthermore, in this study a decrease in the number of neutrophils by 50% at the 1.2 mg/animal dose would seem to be biologically significant. These data, in light of an only 18% decrease in overall WBCs, suggest a fairly specific effect on neutrophils. These data suggest the possibility that repeated use of DHE 45 Nasal Spray may be immunosuppressive.

7. 3-month intranasal maximum tolerated dose study in mice. GLP. Sandoz Study No. T-2963, August, 1994).

The objective of this study was to investigate the toxicity of DHE-45 Nasal Spray in CD-1 mice, including any locally induced toxicity in the respiratory tract, after daily intranasal administration for 13 weeks.

Study Description

Test article

DHE 45 Nasal Spray (Batch #T-21018)

Animals

CD-1 mice, approximately 4 weeks old, M 15-20g, F 15-18g. Bloods were collected from 5 males and 5 females before beginning the study and tested for mouse hepatitis virus, pneumonia virus of mice, Sendai virus, minute virus of mice, mouse polio virus, reovirus type 3 and mycoplasma pulmonis. Full histopathology was also done on these mice. Results showed that the bloods were negative for the specified microorganisms and that there were no pathological abnormalities in animals before the study began.

Treatment Regimen

Each group consisted of 10 males and 10 females for toxicity testing and an additional satellite group of 6 animals per sex per group used for toxicokinetics analysis (blood collection) of Day 1 and Week 13 of the study. The following table shows the dosing regimen for the animals:

Treatment Regimen for the mouse study

Dose Group	Volume of DHE 45 Nasal Spray Administered (µl/nostril)	Dose (mg DHE 45/animal/day)
1	5 x 5 daily (Vehicle)	0
2	5 x 1 daily	0.04
3	5 x 3 daily	0.120
4	5 x 4 daily	0.160
5	5 x 5 daily	0.20

Administration of the test material was by direct instillation of 5 µl droplets into each nasal orifice using a repeat dose BCL Tipmaster pipette for the first 2 weeks and a BCL 5000F pipette for the remainder of the study. Prior to commencement of the 13 week study, all animals underwent a one week predose conditioning period during which a 5 µl droplet of vehicle solution was instilled into each nostril, to accustom the animals to the coming study procedure.

Observations

Observations included clinical symptoms, body weight, food and water consumption, ophthalmoscopy, hematology (Hb, RBC count, MCV, MCHb, WBC and differentials, platelet counts), clinical chemistry, organ weights (adrenals, brain, heart, kidneys, liver, pituitary, prostate, spleen, testes, thymus, lungs, ovaries, thyroid, uterus), macroscopic and microscopic pathology and femoral bone marrow smears as well as nasal cavity pathology. Clinical chemistry included blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, LDH, total protein, albumin, cholesterol, creatinine, calcium, phosphate, total bilirubin, triglycerides, sodium,

potassium, chloride, protein electrophoresis. A full histological examination of all the below listed tissues, with exception of implant site and spinal cord, was undertaken on all animals in the Control and High dose groups. Additionally the respiratory tract tissues were examined from the Low and Intermediate dose groups. Bone marrow smears were not evaluated since hematological results indicated that this was not necessary. The following tissues were examined: abnormal tissues, adrenals, aortic arch, brain, clitoral gland, femur, gall bladder, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, heart, kidneys, pancreas, pituitary, preputial gland, prostate/uterus, sciatic nerve, skin, seminal vesicles, spinal cord, spleen, sternum + rib, submandibular lymph node, submaxillary salivary gland, thigh muscle, thymus, thyroids + parathyroids, tongue, liver, lung + bronchi, mammary gland, mediastinal lymph node, mesenteric lymph node, oesophagus, ovaries/testes, trachea, urinary bladder, nasal cavity, larynx, cervical lymph node, bronchial lymph node. Toxicokinetics were also analyzed.

For a diagram of the various levels of the mouse nasal cavity as defined in this study, please see page 15 of this review.

Results

Mortalities

There were two deaths during the course of the study, a Control male and a Group 4 (0.160 mg/animal) female. Both animals were killed for humane reasons, as the Control male showed severe hair loss and scabbing around the neck from being held during dosing and the Intermediate dose female suffered eye damage during retro-orbital bleeding.

Clinical Symptoms

There were no clinical symptoms of note in the study animals.

Body Weights and Food Consumption

During the first three weeks of the study, Groups 1(Control), 3 (0.12 mg/day), 4 (0.16 mg/day) and 5 (0.2 mg/day) lost a small amount of weight, with the High Dose losing about 6% total body weight. A similar trend was found with female animals, with the High Dose group losing about 3% of their body weight. After three weeks, the animals consistently gained weight at about the same rate in all groups up to the end of the study at 13 weeks.

With respect to food consumption, males demonstrated transiently decreased consumption in Group 3 (17%, Week 8), Group 4 (14%, Week 9) and Group 5 (22%, Week 9). Females also demonstrated transiently decreased food consumption in Group 4 (6%, Week 8) and Group 5 (9%, Week 9). Only females demonstrated an overall decrease in food consumption over the 13 week study, and this decrease was dose-related, with 2%, 7%, 16% and 16% decrease in food consumption in Groups 2, 3, 4, and 5, respectively.

Ophthalmic Evaluations

No changes related to treatment were found.

Hematology

The only hematological findings were a decrease in Hb (5%, High Dose) and RBC (7%, Intermediate Dose Group 4) in males. These findings were dose-related, but no such findings occurred in females.

Clinical Chemistry

Due to the minimal amount of blood that the sponsor was able to collect from the mice, the many of the clinical chemistry data parameters are missing. Also, the data that are present are extremely variable. The only data that may be relevant are the LDH levels in males. LDH levels increased about 50% in Group 3 males, and were increased to a lesser degree in Group 4 (21%) and Group 5 (23%).

Organ Weights

When data were analyzed as organ:body weight ratio, thyroid weights were decreased in both male (25%, High Dose) and female (44-48%, Groups 2-5, respectively) animals. Liver weights were decreased in males (Group 4, 14%).

Macroscopic Pathology

No notable macroscopic findings of increased incidence were seen.

Microscopic Pathology

The only results of note were found in the nasal cavity of the animals. These are enumerated in the following table:

Incidence of Lesions in the Nasal Cavity of Mice in the 3-Month Toxicology Study
Note: 10 animals per sex were examined in each dose group

Findings	Males					Females				
Treatment Grp	1	2	3	4	5	1	2	3	4	5
NASAL CAVITY										
No abnormalities	2	0	0	0	0	0	0	0	0	0
Chronic inflammation in harderian gland										
Level III	2	0	0	0	0	0	0	0	1	0
Level IV	4	5	2	5	3	2	3	5	7	1
Very mild Goblet Cell Proliferation										
Level I	0	2	4	7	7	1	5	5	7	10
Very mild focal cranial nerve fiber degeneration										
Level II	1	2	1	0	0	1	1	0	0	0
Level III	3	2	1	0	0	2	2	1	1	0
Level IV	3	2	2	0	0	4	2	0	1	2
Mild focal cranial nerve fiber degeneration										
Level I	1	0	0	2	0	0	0	0	0	0
Level II	4	3	1	2	1	2	2	1	1	0
Level III	4	5	1	3	1	5	4	3	3	2
Level IV	4	5	2	3	1	4	4	3	3	3
Moderate focal cranial nerve fiber degeneration										
Level II	0	0	1	1	0	0	0	0	1	0
Level III	0	0	1	1	0	0	1	0	1	0
Level IV	0	0	1	1	0	0	1	0	1	0
Mild diffuse respiratory epithelial eosinophilic inclusion(s)										
Level I	0	0	2	4	3	0	2	0	0	1
Level II	0	0	2	4	3	0	2	0	0	1
Level III	0	0	0	2	0	0	0	0	0	0

Moderate diffuse respiratory epithelial eosinophilic inclusion (s)										
Level I	0	1	2	3	3	0	4	6	7	2
Level II	0	1	2	3	3	0	4	6	7	2
Level III	0	0	0	1	1	0	2	3	2	0
Severe diffuse respiratory epithelial eosinophilic inclusion(s)										
Level I	0	0	0	0	0	0	0	3	3	7
Level II	0	0	0	0	0	0	0	3	3	7
Level III	0	0	0	0	0	0	0	3	3	0
Very mild focal respiratory epithelial eosinophilic inclusion(s)										
Level I	0	1	0	0	1	0	0	0	0	0
Level II	2	1	1	0	2	1	0	0	0	0
Mild focal respiratory epithelial eosinophilic inclusion(s)										
Level I	0	4	2	3	1	0	0	0	0	0
Level II	1	5	4	3	1	0	0	0	0	0
Level III	0	1	1	0	0	0	0	0	0	0
Moderate focal respiratory epithelial eosinophilic inclusion(s)										
Level I	0	1	1	0	0	0	4	0	0	0
Level II	0	1	1	0	0	0	4	0	0	0
Level III	0	0	1	0	0	0	2	0	0	0
Very mild focal eosinophilic olfactory inclusion(s)										
Level I	0	1	1	0	0	0	0	0	0	0
Level II	0	1	0	0	0	0	0	2	0	0
Level III	1	0	0	0	0	0	0	0	0	0
Mild focal eosinophilic olfactory epithelial inclusion(s)										
Level I	0	0	0	0	1	0	1	1	1	2
Level II	0	0	0	0	0	0	0	1	1	1
Level III	0	0	0	0	1	0	0	1	0	0
Level IV	0	0	0	0	0	1	0	0	0	1
Moderate focal eosinophilic olfactory epithelial inclusion(s)										
Level I	0	0	0	0	1	0	0	1	2	2
Level II	0	0	0	0	0	0	0	1	1	2
Level III	0	0	0	0	0	0	0	0	1	0
Level IV	0	0	0	0	0	0	0	0	1	0
Very mild focal rhinitis										
Level I	0	1	1	0	0	0	0	1	0	0
Level II	4	1	1	0	0	2	0	0	0	0
Mild focal rhinitis										
Level II	0	0	0	0	0	1	0	0	0	0

Very mild diffuse rhinitis										
Level I	0	7	4	4	6	0	6	3	0	1
Level II	1	3	6	4	5	2	6	3	0	1
Level III	0	0	0	0	0	0	1	0	0	0
Mild diffuse rhinitis										
Level I	0	0	1	6	4	0	4	6	9	7
Level II	0	0	1	6	4	0	4	6	9	7
Level III	0	0	0	0	0	0	2	0	0	0
Moderate diffuse rhinitis										
Level I	0	0	0	0	0	0	0	0	1	2
Level II	0	0	0	0	0	0	0	0	1	2
Very mild focal ulceration										
Level I	0	0	0	0	0	0	0	0	1	0
Mild focal ulceration										
Level III	0	0	0	1	1	0	0	0	0	0
Mild focal squamous metaplasia										
Level III	0	0	0	0	1	0	0	0	0	0
Intraluminal inflammatory exudate										
Level III	0	0	0	0	1	0	0	0	0	0

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 from Group 5, 10/10 females and 9/10 males from Group 4, 9/10 females and 6/10 males from Group 3 and 8/10 females and 7/10 males from Group 2. It was present in 2/10 Control females and in none of the Control males.

Very mild to severe grades of eosinophilic inclusions were present in the respiratory epithelium of almost all animals exposed to DHE 45 Nasal Spray. Very moderate grades of focal eosinophilic inclusions were seen in the olfactory epithelium of 5/10 females from groups 3 and 5 and 4/10 females from Group 4 and 3/10 males from Group 5. Single incidences were present in Group 3 males, in both sexes of Group2, and in both sexes of Group 1.

Very mild Goblet cell proliferation was present in 7/10 males and 10/10 females from Group 5, 7/10 males and 7/10 females from Group 4, in 4/10 males and 5/10 females from Group 3, 2/10 males and 5/10 females in Group 2 and one female Control.

Very mild focal ulceration was found in 1/10 females in Group 4. Mild focal squamous metaplasia was seen in 1/10 Group 5 males.

There was also a high incidence of focal cranial nerve fiber degeneration. The sponsor states that, since 4/10 animals demonstrated mild focal cranial nerve fiber degeneration at Levels II-IV in the Control Group, that this effect is probably due to the intranasal treatment methodology rather than to the administration of the drug. However, moderate focal cranial nerve fiber degeneration also occurred in Group 3 and 4 males and Group II and IV females in the absence of effects in Controls. It is very difficult from these data to determine whether this effect on cranial nerve fibers is due to the physical treatment of the animals or the drug itself.

Reviewer's Comments

As with previous studies in the rat and mouse, these data demonstrate evidence of chronic irritation in the nasal cavity as the result of intranasal administration of DHE 45 Nasal Spray. As in the case of the rat, the mouse data also demonstrated the progressive nature of the nasal cavity effects. A higher incidence of Goblet cell proliferation occurred, in addition to the appearance of focal ulceration and one case of squamous metaplasia. These results, as with those for rat, indicate the potential for these nasal cavity symptoms to be progressive in nature, and serve to again emphasize the importance of the results of the carcinogenicity studies in rats for DHE 45 Nasal Spray by the intranasal route.

Also, as in the case of the 3-month rat study, no NOEL was found for the respiratory epithelial eosinophilic inclusions, the eosinophilic olfactory inclusions, the rhinitis, or the Goblet cell proliferation in the mice. The incidence of focal ulceration (1/10 females, 0.12 mg/day, Level 1; 1/10 males at 0.16 and 1/10 males at 0.20 mg/day, Level II) and squamous metaplasia (1/10 males, 0.2 mg/day) were low. This lack of a NOEL is a source of great concern and is consistent with the rat studies. This means that, in terms of nasal cavity surface area, the Goblet cell hyperplasia, eosinophilic inclusions and rhinitis are occurring at doses of drug in the mouse that ath which exposure to drug level is at least in the same range as the human (see next Section of Toxicokinetics), and that we actually do not know how low of a dose one has to administer before these effects cease to occur.

Toxicokinetics Studies

A satellite group of animals (6/sex/group) were used to measure plasma levels. Plasma levels were determined on Day 1 and Week 13, with bloods collected 30 minutes after the last dose in all cases. The following table shows toxicokinetics data for this study.

Plasma concentrations of DHE at designated sampling time on Week 1 (Day 1) and Week 13 of Dosing

Dose Group (mg/animal/day)	Males Plasma Conc (ng/ml) (SD)	Female Plasma Conc (ng/ml) (SD)
WEEK 1 Control	BQL*	BQL
0.04	24.8 (8.36)	16.3 (19.2)
0.12	15.3 (1.93)	35.1 (45.3)
0.16	7.1 (10.3)	23.2 (11.8)
0.2	27.3 (17.9)	19.3 (15.2)
WEEK 13 Control	BQL	BQL
0.04	18.8 (8.1)	20.2 (17.8)
0.12	25.1 (10.3)	46.2 (35.1)
0.16	35.7 (24.3)	35.2 (9.3)
0.2	35.6 (15.7)	32.6 (10.6)

BQL=Below quantifiable limit (9 ng/ml)

These data demonstrate again the large variability involved in intranasal administration. Values are seen to represent up to ± 100% variability. In this study with the mouse, the plasma levels do not increase linearly with dose and on Week 13 demonstrate a plateau at about 0.12 to 0.16 mg/animal/day. There does not appear to be any accumulation of drug with time nor does there appear to be any major difference in plasma levels with animal gender. The problem of lack of linear relationship between increasing dose and plasma level may be due to the relatively small nasal cavity of the mouse. The sponsor could only administer a maximum of 5 μl liquid at a time, and with this small volume it is likely that not all of the drug remained in the nasal cavity.

Mouse exposure to DHE 45 Nasal Spray in terms of human exposure to the prescribed dose

The following includes comparison of mouse versus human exposure to the drug by three different analytical methods.

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)
Mouse	low dose (0.04 mg)	plasma C_{max} MOUSE receive 20-FOLD> MAN	MOUSE=0.04 mg HUMAN=2.0 mg HUMAN 50-FOLD>MOUSE	MOUSE=.00013 HUMAN=0.0001 HUMAN AND MOUSE IN SAME RANGE
Mouse	high dose (0.2 mg)	plasma C_{max} MOUSE 30-FOLD>HUMAN	MOUSE=0.2 mg HUMAN=2.0 mg HUMAN 10-FOLD>MOUSE	MOUSE=0.0007 HUMAN=0.0001 MOUSE 7-FOLD>HUMAN

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 (not involving nasal cavity histopathology findings), 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).**

Mice receiving the lowest dose, 0.04 mg/day, when compared to humans with respect to plasma levels, demonstrated about 20-fold higher plasma levels than humans receiving the prescribed dose. With respect to the highest dose, 0.2., mice in this 3 month study were exposed to plasma levels of up to about 30-fold higher than humans receiving the prescribed dose of 2 mg/headache. There were no AUC data for the mice in this study.

Total Dose

With respect to the drug side effect found in the nasal cavity, systemic exposure is probably not as relevant as local exposure to the drug in the area of the nasal cavity. One way to compare exposure levels between rat and human is to simply compare the total dose administered to the nasal cavity in the two species.

With respect to the lowest dose, 0.04 mg/day, administered to the mice, the human receiving the prescribed clinical dose of 2 mg per migraine headache would receive about a 50-fold greater exposure in terms of total dose than the mice at this dose. Considering the highest dose administered to the mice, 0.2 mg/animal/day, the human would be exposed to approximately 10-fold higher level of drug in the nasal cavity in terms of total dose.

Dose Per Surface Area of Nasal Cavity (mg/mm^2)

Another, perhaps more accurate, method of comparing mouse and human exposure to DHE 45 in the nasal cavity is to compare the two in terms of drug dose (mg) per nasal cavity surface area (mm^2). This method allows for normalization of the total dose of drug in terms of local surface area to which the drug is being administered. In these terms at the mouse low dose, the human and the mouse would be receiving about the same level of drug exposure. At the mouse high dose, the mouse would be exposed to a drug level about 7-fold greater than the human.

Reviewer's Comments

These mouse data are consistent with the rat data in terms of nasal cavity effects. The mouse data corroborate the rat findings in indicating that the histopathological findings in the nasal cavity are progressive with time. Also, no NOEL was found for Goblet cell hyperplasia, eosinophilic inclusions and rhinitis, which means that we do not actually know how low of a dose will cease to cause these effects. We do know, based on these data, that mice receiving about the same level of exposure to drug in the nasal cavity (based on nasal cavity surface area) as humans administered the prescribed dose experience these chronic inflammatory effects.

Data from this study also further demonstrate the large variability of plasma levels with dose of drug administered intranasal. These data indicate the difficulty of administering a consistent dose with this drug by the intranasal route.

The only other toxicological result worthy of mention is the decrease in thyroid weights. The meaning of this finding is unclear.

The sponsor recommended, based on these variable data, that the mouse not be used as a carcinogenicity model by the intranasal route of administration due to difficulties with consistent dosing. Based on these data, I would agree that the mouse would not be a very good model for carcinogenicity studies by the intranasal route. However, in terms of a model for examining potential carcinogenicity of the drug in target organs other than the nasal cavity using an alternative route of administration to the intranasal route, the mouse is a perfectly acceptable model.

Genetic Toxicology:

So far the sponsor has submitted the following genetic toxicology studies:

Previously submitted:
1. Mutagenicity Evaluation using Salmonella Typhimurium (1983)
2. Micronucleus test for Mutagenic Potential in Mice (1973; non-GLP)
3. Micronucleus Test and Cytogenetic Analysis of Chinese Hamster Bone Marrow Cells for Evaluation of Mutagenic Potential (1973; non-GLP)

Submitted in Amendment dated 1/25/95:
4. Chromosome Aberration in Cells of Chinese Hamster Cell Line V79 (Doc. No. 203-127).
5. Test for the Induction of DNA Repair Synthesis (UDS) in Rat Hepatocyte Primary Cultures (Doc. No. 203-111).
6. Mutagenicity Evaluation in V79 Chinese Hamster Cells (HGPRT-Test) (Doc. No. 203-117).

7. An independent evaluation by
of the entire genotoxicity package to date.

The following have been promised by the sponsor but have yet to be submitted:

1. Mouse Micronucleus test in mice under GLP conditions using current study design (report available April 3, 1995).
2. Human Lymphocyte Assay, In Vitro Test for Chromosomal Aberrations (to replace micronucleus test and cytogenetic analysis in Chinese hamster bone marrow cells; report available May 8, 1995).

Review of individual genetic toxicology studies:

1. **DHE-45: Chromosome Aberrations in Cells of Chinese Hamster Cell Line V79,** GLP, Batch # 83013, done at
October 22, 1986,
sponsor Sandoz Pharmaceuticals.

Study Description:

The test system is the V79 cell line of Chinese Hamster fibroblasts with a doubling time of 12-16 h and plating efficiency of untreated cells of 70-90%. The cells have a karyotype with a model chromosome number of 22. The negative controls are untreated cultures and cultures treated with solvent (DMSO). The positive controls were EMS (ethylmethanesulfonate) without metabolic activation and cyclophosphamide with metabolic activation. Cells were treated for four hours and sampled at 7, 18 or 28h after treatment. The data in the range-finding cytotoxicity study vary all over the place and make it very difficult to determine an appropriate maximum concentration of DHE based on the 50% decrease in mitotic index principle. The sponsor does appropriately evaluate 3 concentrations of drug. The product was used was DHE-45, dissolved in DMSO. The sponsor states that the DMSO was not toxic to the cells. It is unclear from the submission whether or not the DHE-45 includes the caffeine that is in the final preparation in the clinical trials.

Reviewer's Comments:

1. This genetic toxicology study appears to have been done by GLP and to contain most of the desirable characteristics of such a test, including proper timing of cell sampling, appropriate number of drug concentrations tested, presence of positive and negative controls with appropriate results, presence and absence of S9 fraction, appropriate number of cells tested, and appropriate cytotoxicity range-testing prior to the actual mutagenicity testing.

2. The data regarding the preliminary cytotoxicity range-finding vary considerably, making it difficult to determine an appropriate maximum concentration of DHE to include in the study.

3. DHE demonstrated a positive response at the 18 hour time point as follows:

Treatment	Number of cells analyzed	S9	Aberrant including gaps (%)
1. untreated cells	400	-	10 (2.5)
2. untreated cells	400	+	14 (3.5)
3. solvent control	400	-	18 (4.5)
4. solvent control	400	+	18 (4.5)
5. + control (EMS)	200	-	32 (16)
6. + control (cyclophos)	200	+	45 (22.5)
7. DHE 3 μg	400	-	17 (4.25)
8. DHE 8 μg	400	+	13 (3.25)
9. DHE 15 μg	400	-	20 (5)
10 DHE 40 μg	400	+	14 (3.5)
11.DHE 55 μg	400	-	23 (5.75)
12.DHE 105 μg	**400**	**+**	**39 (9.75)**

4. The sponsor acknowledges that these data demonstrate a positive response at the 105 μg concentration. However, they claim that this is also the concentration at which cytotoxicity studies demonstrate about 48% viability, the maximum valid concentration to be used in the studies. They suggest that, under these circumstances, these data should do not constitute a positive for chromosomal aberration. They state that these data should be considered only in light of other genetic toxicology data.

The sponsor's argument that the 48% viability is problematic in determining whether or not the 105 μg concentration of DHE is positive is insufficient to allow one to ignore this value. Although the viability at this concentration may be diminished for the cultures in general, the analysis requires that a total of 400 cells be analyzed at each concentration, and 400 cells were apparently analyzed at the 105 μg dose, with 39% found to contain aberrations. Therefore, I would interpret these data to mean a positive response, and would like to see the assay repeated for confirmation. The sponsor apparently chose not to repeat.

5. Deficits in these data include the fact that the range-finding cytotoxicity data were not submitted, ± S9 fraction was not included at every DHE concentration, and the test was not repeated to confirm the results. In absence of a repeat study or the data from the range-finding cytotoxicity study, it is difficult to determine, based on these data, whether or not the drug actually caused chromosomal aberrations in these cells at the high dose (105 μg), although as explained in #4 above, the sponsor's argument is not sufficient to simply ignore the positive response at the 105 μ concentration of DHE.

2. Test for the Induction of DNA Repair Synthesis (UDS) in Rat Hepatocyte Primary Cultures, GLP (Switzerland), Study # HPC/UDS/A 7/87, Sandoz, LTD, Basle, Switzerland, March, 1988.

Study Description:

The sponsor evaluated 15 concentrations of the drug, from 0.0192 to 300 μg/ml. Primary cultures of rat hepatocytes were treated with the drug. The incorporation of ^{3}H-thymidine was measured by autoradiography. Cytotoxicity prevented the evaluation of the slides treated with 60 and 300 μg/ml, respectively. The test compound, DHE 45 (Batch 86023), was dissolved into DMSO. Positive controls included 2-Acetylaminofluorene and aflatoxin B_1. DHE was cytotoxic at concentrations >24 μg/ml as indicated by the absence of cells suitable to analyze on the slides treated with 60 and 300 μg/ml.

Animals: Hepatocytes were prepared from adult, male KFM:Wist rats (200-300 g).

Dose Selection: The first experiment is performed using the highest soluble concentration and 6 additional doses obtained by dilution in five-fold steps.

Evaluation of the slides: The DNA repair synthesis is quantified by determining the production of silver grains by the decay of ^{3}H-thymidine incorporated into DNA. Positives are determined by the following criteria:

1. A net grain count of the solvent control is ≤5.
2. The positive control chemicals induces UDS according to the criteria given below.
3. A test compound is considered active in the UDS assay if the net grain count is ≥5, and there are at least six grains per nucleus in excess of the concurrent solvent control, at least of one concentration level. The finding is more convincing if the positive value is part of a dose-effect curve. A dose-dependent increase of the number of nuclei with net grain count of ≥5 can provide further confirmatory information. It has to be checked, however, whether the increase in net grain count is due to a higher number of grains in the nucleus or due to a decreased number of grains in the cytoplasm. The latter would indicate a cytotoxic rather than a genotoxic effect.

A test compound is definitely considered positive and thus genotoxic under the conditions of the experiment if the results of the first test are confirmed by the data of the second experiment. Additional information can be obtained rom counting more cells per slide and/or by evaluation of the third series of slides. If necessary a third experiment might be necessary to reach a definite conclusion.

4. A test compound is considered negative and thus non-genotoxic in the hepatocyte UDS test if the net nuclear grain count remains ≤5 at all concentrations, or if it is ≥5, it is not more than 5 grains in excess of corresponding solvent control.

This only holds true, however, if the highest concentration tested is cytotoxic or if limited solubility prevents the use of cytotoxic test compound concentrations. Also the negative findings have to be confirmed in a second, independent experiment.

Results:

Solubility--DHE 45 was perfectly soluble in DMSO up to the highest concentration tested, 30000 μg/ml. No precipitations were observed upon addition of this solution to the treatment medium resulting in a maximal end concentration of 300 μg/ml.

Toxicity--DHE 45 was cytotoxic at concentrations >24 μg/ml as indicated by the absence of cells suitable to analyze on the slides treated with 60 and 300μg/ml, respectively.

Induction of DNA repair synthesis--Net nuclear counts, nuclear counts, and the percentage of nuclei in repair were not notably changed by the treatment with DHE 45 up to the highest non-toxic concentration of 24μg/ml. In the first experiment at 12μg/ml and the second experiment at 24μg/ml the mean net nuclear counts were slightly more positive than controls. However, the increase was small and did not satisfy the criteria for positivity.

Reviewer's Comments:

1. DHE 45 was found to have no DNA repair-inducing potential in rat hepatocyte primary cultures. The test utilized the appropriate positive controls and was done a second time to confirm the results.
2. The product tested here was not the Nasal Preparation containing caffeine.
3. The UDS test is often done by treating the animals with the drug and then removing hepatocytes, preparing and measuring DNA repair synthesis. If I understood correctly, in this submission, the test was done in vitro by incubation of the primary cell culture directly with the drug.

Table 1: Determination of DNA repair in primary cultures of rat hepatocytes treated with DHE 45 1st experiment

Test compound: DHE 45
Solvent: DMSO
Test date: September 10, 1987
Study number: HPC/UDS/A 7/87

Slide No.	Concentration µg/ml	Mean nuclear counts	Mean net nuclear counts ± Standard deviation	Mean cytoplasma counts	%nuclei with net nuclear counts ≥5
25	2-AAF	-	63.3 ±23.3	-	100
26	2.25	-	77.6 ±17.6	-	100
28	Aflatoxin B1	-	68.2 ±13.4	-	100
29	0.02	-	47.0 ±14.2	-	100
55	Solvent	32.2	-4.5 ±6.3	36.7	0
56	DMSO	29.6	-3.4 ±4.9	33.0	0
	DHE 45				
58	0.0192	26.8	-5.9 ±5.7	32.7	4
59	0.0192	28.1	-5.8 ±4.8	33.9	0
61	0.096	29.0	-4.4 ±5.3	33.4	4
62	0.096	25.6	-4.4 ±4.9	30.0	4
65	0.48	26.6	-5.4 ±5.9	32.0	4
66	0.48	30.2	-3.4 ±4.2	33.6	0
67	2.4	29.0	-4.0 ±6.0	33.0	0
68	2.4	27.7	-4.7 ±5.1	32.4	0
70	12	28.1	+1.0 ±6.1	27.1	28
71	12	29.91	-0.3 ±5.0	30.2	8
73	60		not evaluated		
74	60		not evaluated		
76	300		not evaluated		
77	300		not evaluated		

*2-AAF *2-acetylaminofluorene

Table 2: Determination of DNA repair in primary cultures of rat hepatocytes treated with DHE 45 2nd experiment

Test compound: DHE 45
Solvent: DMSO
Test date: December 8, 1987
Study number: HPC/UDS/A 7/87

Slide No.	Concentration µg/ml	Mean *nuclear± counts	Mean net nuclear counts Standard deviation	Mean† cytoplasma counts	nuclei with net nuclear counts >5
32	2-AAF	-	48.3 ±10.8	-	100
33	2.25	-	39.3 ±10.3	-	100
34	Aflatoxin B1	-	19.7 ±9.9	-	92
35	0.02	-	17.2 ±7.6	-	92
64	Solvent	16.5	-2.5 ±3.6	19.0	0
65	DMSO	14.5	-2.5 ±2.6	17.0	0
	DHE 45				
67	0.09375	14.6	-2.7 ±3.9	17.3	0
68	0.09375	14.2	-7.0 ±5.6	21.2	4
70	0.1875	14.6	-3.8 ±3.5	18.4	0
71	0.1875	18.9	-2.7 ±3.6	21.6	0
73	0.375	14.2	-3.0 ±2.9	17.2	0
74	0.375	15.7	-2.5 ±3.6	18.2	0
76	0.75	16.9	-3.4 ±3.1	20.3	0
77	0.75	18.3	-2.1 ±3.0	20.4	4
79	1.5	19.1	-2.0 ±3.1	21.1	4
80	1.5	14.7	-3.8 ±2.7	18.5	0
82	3	16.4	-2.2 ±3.0	18.6	0
83	3	17.9	-1.6 ±3.4	19.5	4
85	6	19.9	-1.7 ±3.4	21.6	0
86	6	16.7	-2.7 ±3.2	19.4	0
88	12	15.4	-3.3 ±2.5	18.7	0
89	12	19.2	-2.7 ±3.9	21.9	8
91	24	18.6	-0.1 ±4.0	18.7	4
92	24	17.9	-0.3 ±3.2	18.2	0

*2-AAF *2-acetylaminofluorene

3. Mutagenicity evaluation in V79 Chinese Hamster Cells (HGPRT-Test). GLP (Switzerland), Study Mut V79 III/87, Sandoz Pharma LTD, Basle, Switzerland. October, 1990.

Study Description:

The purpose of this study was to evaluate DHE 45 for its potential mutagenic activity in V79 Chinese hamster cells in vitro, ± the addition of an S9 fraction. DHE 45 (Batch #86023) was dissolved and diluted in DMSO and the final concentration of DMSO did not exceed 1%. Positive controls included methyl methanesulfonate (absence of S9) and aflatoxin B_1 (presence of S9).

Test Organism: The v79 Chinese Hamster cells were obtained from and cultured in Minimum Essential Medium.

Activation System: The S9 liver homogenate was prepared from at least five 7-9 week old male OFA rats, injected with 500 mg/kg Aroclor 1254 five days before sacrifice.

Solubility and Stability: The DHE 45 was solubilized in DMSO and was stable for 24 hours at room temperature. For the test, DHE 45 was dissolved in DMSO and added to the treatment medium to a final DMSO concentration of ≤1%.

Results:

Toxicity: The effects of S9 concentration on toxicity were determined. DHE 45 was cytotoxic at concentrations above 77.7 μg/ml after 3 hours of incubation. The optimal S9 concentration was determined using a range of 0.03 to 30% final concentration of S9 and concentrations of DHE of 90 and 94 μg/ml DHE. S9 had a detoxifying effect, and considering the data the sponsor chose a final concentration of 10% S9 for testing. In presence of 10% S9 DHE 45 induced cytotoxic effects above 354.5 μg/ml.

Mutagenicity

All mutation frequency values were below 2 X 10^{-5}, considered the highest tolerable value for the solvent controls. The spontaneous mutation frequencies were 0.18 and 0.50 X 10^{-5} in absence of S9 mix and 0.11 and 0.35 X 10^{-5} in presence of S9. The positive controls were appropriate and responses were adequate.

In the absence of S9 mix, the increase of the mutation frequency was from 0.18 X 10^{-6} to 0.42 X 10^{-6} at the highest DHE 45 concentration (94.5 μg/ml) (See Table 8 below). The sponsor states that this could not be repeated in a second experiment, but data in Table 9 seem to argue to the contrary. In Table 9, depicting results of a second experiment, the mutation frequency increases from 0.20X 10^{-6} (at 85.7 μg/ml DHE 45) to 0.57 X 10^{-6} (at 90 μg/ml DHE 45). It is true that at 94.5 μg/ml DHE the mutation frequency drops to zero, but at this concentration the viability of the cells decreases dramatically to 68.7% survivors. However, the problem with this experiment is that DMSO (diluent) alone resulted in an increased mutation frequency to 0.50 X 10^{-6}. Therefore, although the second experiment also suggests an increase in mutation frequency, it is impossible to distinguish whether or not this effect is due to the DMSO.

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Table 8 Determination of the frequency of thioguanine resistant V79 mutants in cultures treated for 3h with DHE 45 in the absence of an S9 rat liver homogenate

Test cpd: DHE 45
Solvent: DMSO
Test date: December 10, 1987
Study number: Mut V79, III/87

Test Parameters	Test Compound (μg/ml)	% Survivors
Survivors 6 days after treatment	DHE 45:	
	94.5	81.2
	75.6	87.1
300 cells/plate seeded	60.5	86.1
	12.1	83.3
	DMSO	100
	MMS 90.0	69.7
Thioguanine resistant colonies 6 days after treatment	DHE 45:	
	94.5	0.42×10^{-5}
	75.6	0.13×10^{-5}
	69.5	0.26×10^{-5}
	12.1	0.05×10^{-5}
	DMSO	0.18×10^{-5}
	MMS 90.0	7.4×10^{-5}

Table 9. Determination of the frequency of thioguanine resistant V79 mutants in cultures treated for 3 h with DHE 45 in the absence of an S9 rat liver homogenate (Experiment #2)

Test Parameters	Test Compound (μg/ml)	% Survivors
Survivors 6 days after treatment	DHE 45:	
	94.5	68.7
	90.0	102.0
300 cells/plate seeded	85.7	88.6
	17.1	87.6
	DMSO	100
	MMS 90.0	72.3
Thioguanine resistant colonies 6 days after treatment	DHE 45:	
	94.5	0.00×10^{-5}
	90.0	0.57×10^{-5}
	85.7	$0.20X\ 10^{-5}$
	17.1	0.17×10^{-5}
	DMSO	$0.50X\ 10^{-5}$
	MMS 90.0	19.0×10^{-5}

Table 10. Determination of the frequency of thioguanine resistant V79 mutants in cultures treated for 3h with DHE 45 in the presence of a 10% S9 rat liver homogenate.

Test Parameters	Test Compound (μg/ml)	% Survivors
Survivors 6 days after treatment 300 cells/plate seeded	DHE 45: 1011.6 809.3 647.4 129.5 DMSO AFB_1 0.8	 87.8 96 92.5 99.2 100 76
Thioguanine resistant colonies 6 days after treatment	DHE 45: 1011.6 809.3 647.4 129.5 DMSO AFB_1 0.8	 0.00 X 10^{-5} 000 X 10^{-5} 0.07X 10^{-5} 0.20 X 10^{-5} 0.11X 10^{-5} 3.59X 10^{-5}

Table 11. Determination of the frequency of thioguanine resistant V79 mutants in cultures treated for 3h with DHE 45 in the presence of a 10% S9 rat liver homogenate (Experiment #2)

Test Parameters	Test Compound (μg/ml)	% Survivors
Survivors 6 days after treatment 300 cells/plate seeded	DHE 45: 809.3 539.5 359.7 239.8 DMSO AFB_1 0.8	 78.7 94.5 77.0 96.0 100 86.1
Thioguanine resistant colonies 6 days after treatment	DHE 45: 809.3 539.5 359.7 239.8 DMSO AFB_1 0.8	 0.15 X 10^{-5} 0.08 X 10^{-5} 0.49X 10^{-5} 0.39X 10^{-5} 0.35X 10^{-5} 5.60X 10^{-5}

Reviewer's Comments:

1. The reviewer agrees that the second study does not corroborate an increased mutational frequency at the highest DHE 45 concentration. However, an increased in mutational frequency does occur between DHE 45 concentrations of 85.7 and 90 μg/ml. The reason this does not corroborate the effect in the first study is because the DMSO control mutational frequency (0.50×10^{-5}) in the second experiment is as high as the maximum mutational frequency attained by 90 μg/ml DHE 45(0.57×10^{-5}), making it impossible to determine whether the increase in frequency is due to the DHE 45 or the DMSO.

2. Overall data do suggest that there is no significant increase in mutation frequency with DHE 45 in the concentration range tested. All mutational frequencies are below the 2.5×10^{-5} frequency the sponsor deems as acceptable. There appears to be no effect in presence or absence of S9.

3. The sponsor chose the DHE 45 and S9 concentrations appropriately by the cytotoxicity criterion.

4. Micronucleus Test for Mutagenic Potential in Mice. NON-GLP. Study MK XV 73, Sandoz Pharmaceuticals LTD, Basle, Switzerland, September 18, 1973.

Study Description:

DHE 45 was examined for its mutagenic potential using the micronucleus test.

Animal species: CD-1 mice of both sexes, males 32-40 g, females 27-32 g.

Drug preparation: DHE 45 was dissolved in a mixture containing 0.2% tartaric acid, 6% ethanol and 5% glucose. The mixture was stable at room temperature during the application period.

Administration: The drug was administered twice intraperitoneally 24 hours apart.

Dose and number of animals:

Acute toxicity screen--10 animals (5 of each sex) were used for the acute toxicity screen. Each dose level (3.2, 5, 8, 12.5, 20, 32, 50, 80, 125, and 200 mg/kg body weight) was administered twice and animals observed for 14 days and LD_5 and LD_{50} determined.

Micronucleus Test--3 test dose levels (20, 40 or 80 mg/kg body weight) were used. The high dose was about 2/3 of the LD_{50} value and the low dose was about 1/3 of the LD_5 value. Controls received the mixture of tartaric acid, ethanol and glucose mentioned above in absence of drug. Each dose level was administered twice. 4 animals (2 of each sex) were treated at each dose level. 4 additional animals (2 of each sex) were added to the high dose group as reserves. Six hours after the last drug administration, animals were sacrificed and bone marrow smears prepared for examination for micronucleated erythrocytes. 500 erythrocytes per animal were analyzed.

Results:

Table 1. Toxicity Screen.

Dose of DHE (mg/kg)	# animals died
3.2	0
5	2
8	0
12.5	0
20	0
32	0
50	0
80	2
125	4
200	10

Estimate of LD values by probit analysis: LD_{50}=118 mg/kg LD_{10}=66 mg/kg

Table 2. Micronucleus Test

Drug dose (mg/kg)	Micronucleated erythrocytes (MEs)
0	4 animals had MEs at percentages of 0.0, 0.0, 0.6 and 0.2 mean=0.2
20	4 animals had MEs at the percentages of 0.2, 0.0, 0.4 and 0.0 (mean=0.15)
40	4 animals had MEs at the percentages of 0.0, 0.2, 0.0 and 0.0 (mean=0.05)
80	4 animals had MEs at the percentages of 0.2, 0.4, 0.0 and 0.4 (mean=0.25)

Additional information:

A negative control study performed by the sponsor using 100 CD-1 mice revealed the frequency of micronucleated erythrocytes (MEs) in untreated mice to be in the range of 0.0-0.7% (mean=0.111%) and 99% of the animals were in the range of 0.0-0.4%. From these data the sponsor chose to designate a frequency for MEs of 0.4% as the upper limit of normal for individual animals and of 0.3% as normal for individual groups.

Reviewer's Comments:

1. This study did not use sufficient animals. It is appropriate to use at least 5 animals per sex per group.
2. No positive control was included in the study, and therefore it is unknown whether or not the procedures used in the test were adequate to detect a positive response. Appropriate positive controls would include ethylmethanesulphonate, ethylnitrosourea, mitomycin or cyclophosphamide.
3. The sponsor designated 0.3% as the upper limit of normal for frequency of MEs for an individual group of animals. At 80 mg/kg DHE 45 a mean frequency of 0.4% was found. Also, one of the controls exhibited a higher ME frequency (0.6%^) than the sponsor's designated individual animal upper limit of 0.4%. With data such as this and in the absence of sufficient animals or appropriate positive controls, these data are difficult to interpret.

4. The sponsor has promised to provide data from a Mouse Micronucleus test done under GLP conditions, data to be received by April 3, 1995 to replace this invalid and non-GLP test from 1973.

5. Micronucleus test and cytogenetic analysis of chinese hamster bone marrow cells for evaluation of mutagenic potential. NON-GLP. Study CH VI, Sandox Pharma LTD, Basle, Switzerland, October 2, 1973.

Study Description:

To examine a possible mutagenic effect of DHE 45, a chromosome examination of bone marrow cells and the micronucleus test of bone marrow erythrocytes were carried out using adult Chinese hamsters of both sexes. In the chromosome test, three dose-levels were given twice i.p. (0, 20, and 40 mg/kg) with an interval of 24 hours. 16 animals were used for the control; 6 and 14 animals for the 20 and 40 mg/kg body weight groups, respectively. 6 animals of each dose level were used to score the chromosome aberrations. The micronucleus test was performed using four dose levels (2X I.P., 24 hours apart) (0, 12, 25 and 50 mg/kg body weights. Four animals were used for each dose level.

Results:

Toxicity Screen

The toxicity of DHE 45 showed a biphasic dose effect relationship. At 20 and 32 mg/kg none of the animals died, whereas in the range of 2 to 12.5 mg/kg and at concentrations >50 mg/kg at least one of four animals treated per dose died. The concentrations chosen for the mutagenicity experiments were all in the upper part of the dose effect curve, i.e. 12.5, 25 and 50 mg/kg for the micronucleus test and 20 and 40 mg/kg for the metaphase analysis.

Chromosome Examination

Control groups: Six animals gave the following percentages of metaphases with chromosomal aberrations: 2.0, 2.0, 0.0, 0.0, 0.0, and 0.0 (mean=0.67).

20 mg/kg/group: Six treated animals gave the following percentages of metaphases with chromosomal aberrations: 2.0, 2.0, 0.0, 0.0, 0.0 and 0.0 (mean=0.67).

40 mg/kg/group: Six treated animals gave the following percentages of metaphases with chromosomal aberrations: 2.0, 0.0, 0.0, 0.0, 0.0 and 0.0 (mean=0.33).

Micronucleus Test

Dose (mg/kg)	Micronucleated erythrocytes (%)
0	4 animals=0.2, 0.2, 0.3, and 0.2 (mean=0.23)
12.5	4 animals=0.1, 0.1, 0.1, and 0.3 (mean=0.15)
25	4 animals=0.2, 0.1, 0.1 and 0.0 (mean=0.1)
50	4 animals=0.2, 0.2, 0.2 and 0.2 (mean=0.2)

The sponsor concluded that based on these data DHE 45 is not clastogenic.

Reviewer's Comments:

1. This study was not done under GLP guidelines.
2. The study lacks the recommended number of animals per sex per group (5).
3. No positive controls are included in the study, and it is therefore impossible to determine whether or not the test was capable of detecting a positive response. Therefore, the data are meaningless.
4. The sponsor has promised to complete a human lymphocyte assay done under GLP guidelines to replace this NON-GLP study.

6. Mutagenicity evaluation using Salmonella typhimurium. GLP (Switzerland). Study Mut.Bakt. 20/83. DHE 45, Sandoz Pharmaceuticals LTD. Basle, Switzerland, May 1, 1983.

Study Description

The objective of the study was to evaluate the DHE 45 compound for genetic activity in Salmonella typhimurium in vitro with and without the addition of a mammalian metabolic activation system.

Test compound

Batch 82003 of drug was diluted into DMSO. Concentrations used were 200, 1500, 2000, 5000, 15000 and 20000 µg/ml.

Positive Controls

Positive controls included 2-aminoanthracene, benzo(a)pyrene, MNNG, 9-aminoacridine and 2-nitrofluorene.

Indicator microorganisms

Indicator microorganisms included Salmonella typhimurium strains TA1535, TA1527, TA1538, TA98 and TA100.

Procedure

The test was carried out twice to confirm results. The numbers of revertant colonies on each plate were counted using a "Fisher Count All model 600" colony counter. Two or three plates per DHE 45 concentration were used.

Results

Results are shown in Tables 1 and 2 below.

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Table 1. Summary of plate test results with liver homogenate.
Test compound: DHE 45
Solvent: DMSO
Test dates: April 21, 1983 and April 28, 1983.

Test compound and conc (μg/plate)	Mean Number of revertants per plate				
	TA1535	TA1537	TA1538	TA98	TA100
First Experiment					
Solvent	21	16	47	51	120
DHE 45					
20	17	18	49	60	121
200	21	18	54	61	106
2000	17F	15F	62	70	B
2-AA*					
3	375	–	1757	1908	–
2-AA					
10	–	124	–	–	1307
Benzo(a)pyrene					
3	–	–	–	488	–
Second Experiment					
Solvent	15	28	36	53	97
DHE 45					
150	13	34	49	63	95
500	18	32	50	71	101
1500	20	22	64	74	50
2-AA					
3	315	–	229	1038	–
2-AA					
10	–	107	–	–	757
Benzo(a)pyrene					
3	–	–	–	321	–

*AA=Animoanthracene
– = not tested
B = small colonies or no background growth
F = reduced background lawn growth

Table 2. Summary of plate test results without liver homogenate.
Test compound: DHE 45
Solvent: DMSO
Test dates: April 21, 1983 and April 28, 1983.

Test compound and conc (μg/plate)	Mean Number of revertants per plate				
	TA1535	TA1537	TA1538	TA98	TA100
First Experiment					
Solvent	46	19	27	29	108
DHE 45					
20	34	13	28	38	118
200	41	9	25	32	105
2000	13F	6	30	24	B
2-AA*					
3	–	–	198	307	–
2-AA					
10	1916	–	–	–	1350
Benzo(a)pyrene					
3	–	749	–	–	–
Second Experiment					
Solvent	27	17	24	31	120
DHE 45					
150	31	17	24	36	113
500	34	19	29	35	113
1500	19	12	23	34	48
2-AA					
3	–	–	160	242	–
2-AA					
10	2284	–	–	–	2229
Benzo(a)pyrene					
3	–	576–	–	–	–

*AA=Aminoanthracene
– = not tested
B = small colonies or no background growth
F = reduced background lawn growth

Toxicity

DHE 45 (±S9) was found to be slightly toxic at the two maximal concentrations of 2000 μg/ml (strains TA1535, TA 1537 and TA100) and 1500 μg/ml (strain TA100).

Mutagenicity

The positive control compounds showed a positive response, indicating that the assay could appropriately detect revertants. None of the five strains tested showed an increase in the number of revertant colonies with DHE 45 treatment that would constitute a positive response.

Conclusions

The sponsor concluded that the compound DHE 45 did not show evidence of mutagenic potential under the above conditions.

Reviewer's Comments

1. At present we would ask for at least five concentrations of drug to be tested with approximately half log intervals between test points.

2. The fact that at 2000 μg/ml for strains TA1535 (±S90), TA1537 (+S9) and strain TA100 (±S9) there is toxicity makes it impossible to determine whether or not the response would have been positive. Certainly with strain TA1535, both with and without S9, the response increased with dose of DHE 45 at 20 and 200 μg/ml. The toxicity at 2000 μg/ml may be masking a positive response. Also in the second experiment without liver homogenate (Table 2), responses with strain TA1535 decreased at 1500 μg/ml drug. These data suggest toxicity at this dose as well, although the sponsor does not indicate this to be the case.

3. The sponsor should submit the individual data for these studies, as this would allow a more detailed analysis of the results. With a data summary only, it is impossible to determine whether or not the decreased revertant values at the high dose are due to toxicity in all plates or to toxicity in a single plate in each group. Analysis of those individual data would allow the reviewer to determine whether or not additional testing should be required.

Reviewer's Summary of Genetic Toxicology Data:

Six genetic toxicology studies have been submitted with the NDA to date, three in the original submission and three in an Amendment received on 1/25/95. Two of these six studies were inadequate due to a lack of positive controls and the fact that they were not done under the GLP guidelines. The two inadequate studies were in the group submitted with the original NDA and were the following:

1. Micronucleus test for mutagenic potential in mice.
2. Micronucleus test and cytogenetic analysis of Chinese Hamster bone marrow cells for evaluation of mutagenic potential.

The sponsor has promised to send GLP studies to replace these inadequate ones. These promised studies include the following:

1. Mouse micronucleus test using current study design (due April 3, 1995).
2. Human lymphocyte assay, In Vitro test for chromosomal aberrations (due May 8, 1995).

Compliance with recommended requirements for genetic toxicology testing

When the sponsor submits results of these two additional genetic toxicology studies, they will have submitted sufficient studies to meet the recommended requirements. They will have included a bacterial gene mutation assay (Ames test), a mammalian gene cell mutation assay (chromosomal aberration assay in cells of Chinese Hamster Cell line V79, mutagenicity evaluation in V79 Chinese Hamster Cells (HGPRT-Test), human lymphocyte assay) and an in vivo mutagenicity test (mouse micronucleus).

Adequacy of study results

With respect to study results, data from the chromosomal aberration test in cells of Chinese Hamster cell line V79 suggest that the drug may be clastogenic at the highest concentration (105 μg). The sponsor argues that these results are clouded by the fact that this concentration is also reported by the sponsor to result in toxicity (only 48% cell viability) and the sponsor's contention is that these data should be evaluated in terms of the other genotox studies. However, this argument is insufficient to negate this positive finding, because the same number of cells (400) were analyzed at each concentration of DHE, thus normalizing for loss of cells in each culture due to decreased viability. The

sponsor chose not to repeat the test for confirmation of the results. The test for induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures indicated that no unscheduled DNA repair was induced by DHE 45. Results of the mutagenicity evaluation in V79 Chinese Hamster cells (HGPRT test) indicated no increase in mutation frequency, although a high value with the DMSO Control in the second experiment was unfortunate. The mouse micronucleus test and micronucleus test and cytogenetic analysis of Chinese Hamster bone marrow cells for evaluation of mutagenic potential test were very old (1973) , non-GLP and did not contain sufficient numbers of animals or positive controls, and therefore were invalid studies. The mutagenicity evaluation using Salmonella typhimurium also demonstrated negative results with respect to DHE 45 at two concentrations. However, the caveat with this study was that, at the high dose in the TA1535 strain (±S9), it is impossible to determine whether or not a positive response occurred due to toxicity.

In conclusion, one study indicates that DHE 45 is not mutagenic, one study demonstrates that the drug does not induce unscheduled DNA repair, one study raises the possibility of clastogenicity (toxicity and the lack of a repeat study cloud the results), two studies are invalid, and the bacterial gene mutation assay data are also problematic due to toxicity at the high concentration range where values appeared to be on the increase.

In order to render a final decision on the potential mutagenicity of the drug, we will have to review the data from the two additional studies that have been promised by the sponsor (mouse micronucleus and human lymphocyte assays). Additionally, the sponsor should submit the individual data from the bacterial gene mutation assay so the reviewer can determine whether or not the assay is sufficient as it stands or needs repeating.

Reproductive Toxicology :

INTRANASAL REPRODUCTIVE TOXICOLOGY STUDIES (submitted in Amendment received 7/14/94)

1. Dose range-finding study on DHE-45 nasal spray in pregnant rabbits. GLP. Sandoz Project T-2886, Sandoz Pharmaceuticals Corp, East Hanover, New Jersey, October 7, 1993.

The purpose of this study was to determine lethal and/or toxic doses of DHE-45 Nasal spray in pregnant rabbits and to detect any embryo and/or fetal effects following intranasal administration of the test article during the period of major organogenesis from Days 6 to 18 of gestation.

Study Description

Lot/Batch #T-21018 of compound DHE-45 Nasal Spray was used as the test article for this experiment. The formulation consisted of a 4 mg/ml solution of dihydroergotamine mesylate in DHE-45 Nasal Spray Placebo (a solution of 50 mg dextrose, USP, and 10 mg caffeine anyhdrous, USP, made up to 1 ml with Water for Injection, USP). The test system included thirty New Zealand White (SPF) female rabbits (9 months old) obtained from 12 males, about 11 months old were used for mating. Each female was mated with two bucks, The day on which successful mating occurred was considered Day 0 pc (post coitus) or day 0 of gestation for each doe.

Drug was administered intranasal to the female rabbits from Days 6 to 18 of gestation using a micropipette with disposable tapered polyethylene pipet tip. Control rabbits received the vehicle at 1200μl/day. Depending on the dose level, each animal received from three to nine 50-μl droplets per nostril per dosing session for one or two dosing sessions per day. There was an approximately 2-hour interval between each dosing session. Drug was administered to 5 females per group as follows:

Dosing schedule: Dose group (mg/rabbit/day)	# of 50 µl droplets per nostril	Total volume administered (µl/rabbit/day)
Control (0)	6 X 2 daily	1200
Low (1.2)	3 X 1 daily	300
Low-mid (2.4)	6 X 1 daily	600
Mid (3.6)	9 X 1 daily	900
Mid-high (4.8)	6 X 2 daily	1200
High (6.0)	7 or 8 X 2	1500

Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16, 18 and 20 pc. Plasma concentrations of DHE were measured for all five females from each group on Day 19 pc, 24 hours after the first daily dose on Day 18 pc. Female animals were observed for mortality, clinical signs, and maternal body weight. Fetal data were collected as lethality, resorptions, and abnormalities.

Results

Treatment-related clinical symptoms (sneezing, lacrimation, salivation, gasping, audible nasal breathing, aggressiveness) were most predominant at doses ≥4.8 mg/day. A slight body weight loss was seen in the pregnant animals during the first half of the treatment period (Days 6-12 pc) at the 3.6 (-1 to -2% body weight), 4.8 (-2% body weight) and 6.0 mg/day doses (-2% body weight). No drug-related increase in embryo lethality was seen at any dose. The incidence of fetal resorptions was increased slightly at the 2.4 (32% vs 10% controls) and 4.8 (27% vs 10% controls) mg/day doses, but this was not dose-related and the highest dose (6.0 mg/day) had only 13% resorptions (see Table 1, Fetal Data below). The only fetal observations noted at maternal necropsy were 1) dome-shaped head (hydrocephaly) in one fetus at 1.2 mg/day and one at 4.8 mg/day, 2) a dead fetus at 4.8 mg/day and 3) focal areas of subcutaneous hemorrhage or congestion in one fetus at 2.4 and one at 4.8 mg/day. These effects were not considered to be drug-related.

With respect to plasma concentrations, the average DHE plasma concentration obtained 24h after dosing on Day 18 pc were 65.0, 2021, 2317, 2908 and 4462 pg/ml following doses of 1.2, 2.4, 3.6, 4.8 and 6.0 mg/day, respectively. Due to the substantial variability of the plasma levels, data indicated that plasma levels only actually increased at doses up to about 2.4 mg/day (600 µl/day) beyond which no further increase in DHE was observed (See Table 2 below). The sponsor attributes this to the limited capacity of the nostrils to contain the intranasal dose.

Table 1. Fetal Data.

Characteristics of Pregnancy and Fetal Data

Dose (mg/day)	0	1.2	2.4	3.6	4.8	6.0
Fertility (%)	100	100	100	100	100	100
# Pregnant/# Mated	5/5	5/5	5/5	5/5	5/5	5/5
# Dead Pregnant	0	0	0	0	0	0
# of Females that Aborted	0	0	0	0	0	0
# Dead Non-Pregnant	0	0	0	0	0	0
# Dead-Accidental	0	0	0	0	0	0
% Resorptions in Dead Females	-	-	-	-	-	-
# Live Pregnant Does on Day 20	5	5	5	5	5	5
Mean # Implantations	8.2	9.0	8.8	8.0	9.2	9.4
Total Resorptions	4	4	14	4	16	6
Mean # Resorptions	0.8	0.8	2.8	0.8	3.2	1.2
% Resorptions and Dead Fetuses	10%	9%	32%	10%	37%	13%
Mean # Viable Fetuses	7.4	8.2	6.0	7.2	5.8	8.2
Mean # Dead Fetuses	0	0	0	0	2.2	0

Table 2. Plasma Concentration of DHE at 24 hours after initiation of day 18 pc dosing in control and dosing groups.

Dose (mg/day)	Total volume of dose (μl/day)	Number of 50 μl droplets per nostril	Plasma concentration Mean±S.D. pg/ml
0 (Control)	1200	6	836±557
1.03	300	3	65.0±145
2.06	600	6	2021±2440
3.09	900	9	2317±2220
4.11	1200	6 (X2)	2908±3100
5.14	1500	7 or 8 (X2)	4462±5470

Conclusions

The limiting factor in selection of dose levels for a Segment II rabbit teratology study appeared to be maternal toxicity, as determined by weight loss, at dose levels of 3.6, 4.8 and 6.0 mg/rabbit/day. Also, plasma level did not appear to increase with dose above 2.4 mg/rabbit/day (600μl/day). There was no evidence of greater embryo/fetal than maternal susceptibility to DHE 45 Nasal Spray. Therefore, the sponsor proposed a high dose of 3.6 mg/rabbit/day which should induce some maternal toxicity, along with doses of 0.4 and 1.2 mg/rabbit/day as additional lower doses.

Dose in Reproductive Toxicology Study in Rabbits Relative to Recommended Human Dose

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. Female rabbits received up to a maximum of 6 mg/rabbit/day during from Day 6-Day 18 of gestation. In terms of dose, this is a maximum of a 3-fold higher dose than that to be administered to the patient. In terms of plasma C_{max}, 6 mg/rabbit would give about 2-4000 pg/m (plateau probably at about 2500 pg/ml), whereas an intranasal dose of 2 mg in the human resulted in plasma C_{max} of about 1100-1300 pg/ml. Therefore, by plasma C_{max}, the female rabbits probably received at least 2-fold higher plasma C_{max} levels of DHE 45 by the intranasal route than in humans with the prescribed human dosing regimen.

2. Dose-range finding study of DHE 45 Nasal Spray in pregnant rats. GLP. Study Sandoz Project T-2920, Sandoz Pharmaceuticals Corporation, East Hanover, New Jersey, December 2, 1993.

Study Description:

The purpose for this study was to determine lethal and/or toxic doses of DHE 45 Nasal Spray in pregnant rats and to detect any embryo and/or fetal effects, following intranasal administration of the test article during the period of major organogenesis from Days 6 to 15 of gestation.

Test Article: Batch #T21018 of DHE 45 Nasal Spray in vehicle of dextrose, caffeine and Water for Injection, USP.

Animals: Charles River-CD Sprague Dawley rats, 13 weeks old, 48 females for study, 25 males for breeding.

Dosing schedule:

Dose Group	DHE 45 Nasal Spray Dose Level (mg/rat/day)
Control	0
Low	0.24
Low-Mid	0.48
Mid	1.2
Mid-High	1.44
High	2.0

Mating

Each female was mated with one male. Day of successful mating was considered Day 0 pc (post coitus) or Day 0 of gestation for each female.

Drug Administration

Individual animal doses of test article were administered daily (to females only) from Days 5 to 15 via intranasal instillation using a micropipette with a disposable polyethylene pipette, with Control rats receiving vehicle at a volume equal to the high-dose group. Dosing was as follows:

Dose Group (mg/rat/day)	# of 10 µl Droplets per Nostril	Total Volume Administered (µl/rat/day)
Control (0)	5 x 5 daily	500
Low (0.24)	3 x 1 daily	60
Low-Mid (0.48)	3 x 2 daily	120
Mid (1.2)	3 x 5 daily	300
Mid-High (1.44)	4 or 5 x 4 daily	360
High (2.0)	5 x 5 daily	500

Observations:

The uterus was excised intact, weighed, and dissected to examine contents for live, dead and resorbed fetuses. Maternal body weights, gravid uterine weights, and food consumption were also determined.

Toxicokinetics:

Blood was collected from each female on Day 15 pc prior to each of the one, two, four or five dose administrations. Blood collection was then continued at 1, 3, 5, 8 and 24 hours following the last dose. Blood was collected from the tail vein using 25 gauge hypodermic needle.

Results:

Clinical Observations and Mortality

No animals died during the study. The following symptoms were observed at all dosing levels, including Controls: red substance from/on nares, rales, salivation immediately post-dose and gasping. A slight decrease in body weight (-1 to -2%) was seen at the two highest doses (1.44 and 2 mg/rat/day, respectively) between days 6-9 pc. **No differences were seen with food consumption. No effects were seen on fertility or embryo/fetal lethality at any of the doses tested.** (See Table below).

Table. Fetal Observations.

Dose (mg/day)	0	0.24	0.48	1.20	1.44	2.00
Fertility (%)	75%	100%	88%	88%	100%	75%
# Pregnant/# Mated	6/8	8/8	7/8	7/8	8/8	6/8
# Dead Pregnant	0	0	0	0	0	0
# Dead Non-Pregnant	0	0	0	0	0	0
# Live Pregnant Dams on Day 16 pc Necropsy	6	8	7	7	8	6
Mean # Implantations	18.7	15.6	19.1	17.0	17.3	18.7
Total # Resorptions	7	8	11	9	12	8
Mean # Resorptions	1.2	1.0	1.6	1.3	1.5	1.3
% Resorptions and dead fetuses	6%	6%	8%	8%	9%	7%
Mean # Viable Fetuses	17.5	14.6	17.6	15.7	15.6	17.3
Total # Dead Fetuses	0	0	0	0	1	0

Toxicokinetics

The drug was absorbed rapidly by the intranasal route, with t_{max} occurring on the average of 1 hour. C_{max} values are dependent on which part of dose is chosen for T=0 due to the dosing regimen, in which a varying number of intranasal administrations were given over time. Probably, the AUC values are the most valid parameter to examine, and AUC increased in a linear fashion with dose, with the exception of the 1.03 mg/rat/day dose. The sponsor states that "bioavailability" increased with dose, but they actually did not calculate bioavailability per se. This would have been a helpful parameter to have determined.

Dose/plasma concentration with respect to human dosing

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 100-1300 pg/ml (1.1-1.3 ng/ml). This dosing regimen also resulted in AUC of about 5.7 ng.h/ml. In this rat reproductive toxicology dose-ranging study, rats received DHE 45 intranasal at an AUC of up to 164.75 ng.h/ml (C_{max} of 69 ng/ml, but not altogether valid due to dosing scheme). The dose (1.7 mg/rat/day, HD) is in the range of that received by humans, but the AUC (164.75 ng.h/ml) is about 28-fold higher than that received by the clinical dosing regimen.

Plasma concentrations of DHE 45 in rats in study T-2920

Dose of DHE 45 (mg/rat/day)	N	AUC (ng.h/ml)	C_{max} (ng/ml)	t_{max} (h)
0	6	BLQ	BLQ	BLQ
0.21 (salt=0.24)	8	20.525	3.30	1
0.41 (salt=0.48)	7	45.529	3.985	8
1.03 (salt=1.2)	7	30.577	3.586	1
1.23 (salt=1.44)	8	79.569	10.830	1
1.71 (salt=2.0)	6	164.750	69.038	1

BLQ=below quantifiable level

Human PK data for DHE 45 Nasal Spray

Route	Dose (mg)	C_{max} (ng/ml)	t_{max} (h)	AUC (ng.h/ml)	$t_{1/2}$ (h)	Vd_{ss} (L)	f(%)
Nasal	1(n=55) 2(n=63) 4(n=15)	0.73 1.2 2.2	0.9 0.8 0.8	3.6 5.7 9.3	combined average=10	combined average= 796	combined average= 32%

Choice of dose for the Segment II teratology study in rats

The only limiting factor in selection of dose level for a Segment II teratology study was minimal maternal toxicity (seen as decreased weight) at dose levels of 1.2, 1.44 and 2.0 mg/rat/day. No adverse effects except for maternal clinical observations were found at 0.24 and 0.48mg/rat/day. Therefore, the high-dose level of DHE 45 Nasal Spray chosen for the Segment II study was 1.44 mg/rat/day, which should induce evidence of maternal toxicity, and 0.48 and 0.16 mg/rat/day as lower doses.

Reviewer's Comments:

No serious adverse effects were observed in this study, and the reviewer agrees with the sponsor's choice of dose for the Segment II study. 1.44 mg/rat/day should give AUC of about 79 ng.h/ml, which is about 13-fold higher than the AUC (5.7 ng.h/ml) for the human dose of 2 mg. This provides a reasonable margin of safety.

3. Investigation of teratogenic potential of DHE 45 Nasal Spray in rabbits--Segment II Study. GLP. Sandoz Pharmaceuticals Corp., East Hanover, New Jersey, Study #2929, May 12, 1994.

The purpose of this study was to detect possible effects on developing rabbit fetuses after intranasal administration of DHE 45 Nasal Spray to pregnant rabbits at doses of 0.4, 1.2 or 3.6 mg/rabbit/day on Days 6 through 18 of gestation.

Study Description

Test Article

Batch #T-21018 of DHE 45 Nasal Spray was used. The formulation consisted of a 4 mg/ml solution of DHE mesylate in Nasal Spray Placebo (solution of 50 mg dextrose, USP, 10 mg caffeine, anhydrous, USP, made up to 1 ml with Water for Injection, USP.

Animals

Seventy-seven New Zealand White (ELITE-NZW) female rabbits (4-5 months old) were obtained from 12 males, same strain, source, age were obtained for mating. 4 animals per group (1 control) were included in a satellite group for PK studies. 16 females per group were mated.

Dosing

Dose levels were chosen from previous dose-ranging reproductive study in rabbits. Individual animal doses were administered daily (to females only) from Days 6 to 18 pc via intranasal instillation using a calibrated 50 µl pipette with disposable tapered polyethylene pipet tip. Control rabbits received vehicle at a volume equal to that received by the high-dose group.

Animal Dosing

Dose Group	DHE 45 (mg/rabbit/day)	Number of 50 µl droplets per nostril	Total volume administered (µl/rat/day)
Control	0	4 or 5 x 2 daily	900
Low	0.4	1 x 2 daily	100
Mid	1.2	3 x 1 daily	300
High	3.6	4 or 5 x 2 daily	900

Observations

Clinical observations, body weights, mortality, fetal external exam, fetal visceral exam, fetal skeletal exam and toxicokinetics were examined.

Results

Clinical observations and female mortality

Compound-related clinical observations occurred, mainly in the high dose (3.6 mg/rat/day) as follows: sneezing, rales, salivation, gasping, audible nasal breathing, aggressiveness, struggling during dosing. No macroscopic findings were detected that were compound-related.

Maternal body weights

A slight, transient decrease in mean body weight gain was seen in pregnant high-dose animals was seen, with most pronounced effect during the early part of the period (Days 6 to 9 pc) when body weight loss of approximately 1% was observed. Subsequent values during post-treatment period showed a recovery.

Maternal fertility and reproductive performance

Group	Control	Low	Mid	High
DHE 45 (mg/day)	0	0.4	1.2	3.6
No. of animals mated	16	16	16	16
No. pregnant	16	16	15	15
No of females that aborted	1	0	0	1
No. of females that delivered early	1	0	0	0
Maternal mortality	0	0	0	0
Fertility index[1] (%)	100	100	94	94
Gestation index[2] (%)	100	94	100	100

fertility index=percent matings resulting in pregnancy
gestation index=percent pregnancies resulting in litters with one or more viable fetuses at scheduled necropsy

There were no effects on maternal mortality, fertility or reproductive performance.

Fetal Data at Cesarean Section (See Table IV below)

There were no treatment-related effects on implantations, resorptions, or number of dead fetuses. A slightly larger decrease in the number of viable fetuses in the low-dose group reflects the slightly higher occurrence of resorptions in this group due mainly to a single dam with eight resorptions and no viable fetuses. This effect was not dose-related. There were also no treatment-related effects in fetal body weight or sex ratio.

TABLE 4

SUMMARY OF MATERNAL AND FETAL DATA AT CESAREAN SECTION

DOSAGE		0 MG/DAY	0.4 MG/DAY	1.2 MG/DAY	3.6 MG/DAY
Females Mated	N	16	16	16	16
Pregnant	N	16	16	15	15
	%	100	100	94	94
Aborted	N	1	0	0	1
Premature Births	N	1	0	0	0
Female Mortality	N	0	0	0	0
Pregnant at C-section	N	16	16	15	14
Dams with Viable Fetuses	N	14	15	15	14
Dams with all Resorptions	N	0	1	0	0
Corpora Lutea	N	132	144	148	141
	MEAN	9.4	9.0	9.9	10.1
	S.D.	1.8	1.3	2.8	1.5
Implantation Sites	N	114	128	132	116
	MEAN	8.1	8.0	8.8	8.4
	S.D.	2.2	1.3	2.5	3.1
Preimplantation Loss	%	13.6	11.1	10.8	16.3
Postimplantation Loss	%	12.3	23.4	17.4	16.9
Dead Fetuses	N	1	3	5	0
	%	0.9	2.3	3.8	0.0
Resorptions, total	N	13	27	18	20
	%	11	21	14	17
	MEAN	0.9	1.7	1.2	1.4
	S.D.	1.5	2.2	1.3	1.7
Early Resorptions	N	10	18	8	11
	%	8.8	14	6.1	9.3
	MEAN	0.7	1.1	0.5	0.8
	S.D.	1.5	2.0	0.8	1.3
Late Resorptions	N	3	9	10	9
	%	2.6	7.0	7.6	7.6
	MEAN	0.2	0.6	0.7	0.6
	S.D.	0.6	1.1	1.1	1.4

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

TABLE 4

SUMMARY OF MATERNAL AND FETAL DATA AT CESAREAN SECTION

	DOSAGE	0 MG/DAY	0.4 MG/DAY	1.2 MG/DAY	3.6 MG/DAY
Viable Fetuses	N	100	96*	109	96
	%	98	77	83	83
	MEAN	7.1	6.1	7.3	7.0
	S.D.	2.7	2.0	2.3	2.7
Viable Male Fetuses	N	56	51	67	45
	%	56	52	61	46
Live Fetal Body Weight (g)	MEAN	50.2	50.6	48.7	48.9
	S.D.	6.6	4.3	4.3	6.4
Male Fetuses	MEAN	50.1	51.7	49.0	50.5
	S.D.	7.0	3.8	4.9	6.2
Female Fetuses	MEAN	49.4	50.0	48.6	47.3
	S.D.	6.6	5.3	5.2	6.9

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

Fetal Examinations

External Examinations: Dome-shaped head (hydrocephaly) occurred in 2 of 101 low-dose fetuses, and unilateral carpal flexure was seen in 2 of 101 low-dose fetuses examined. No treatment-related effects were seen.

Visceral Examinations: 4 of 101 fetuses demonstrated internal hydrocephaly in the low dose group, but the effect only in this group and was therefore not dose-related. 1 of 101 fetuses also exhibited cleft palate and slight dilatation of lateral brain ventricle, but again only in the low dose group. Therefore, there were no treatment-related effects.

Skeletal Examinations: Malformations: There were a number of fetal skeletal variations as enumerated in the following table:

Effect	Dosage (mg/day)	0	0.4	1.2	3.6
Litters evaluated		15	15	15	14
Fetuses evaluated		102	95	107	97
Live		102	95	107	97
Dead		0	0	0	0
Hyoid body/incompletely ossified					
Fetal incidence	N	13	14	14	21
	%	13	15	13	22
Litter incidence	N	10	7	7	8
	%	67	47	47	57
Skull-one or more bones incompletely/not ossified					
Fetal incidence	N	13	16	15	22
	%	13	17	14	23
Litter incidence	N	10	8	8	8
	%	67	53	53	57
Forelimb digit V-middle phalanx not ossified					
Fetal incidence	N	0	4	0	20
	%	0	4.2	0	21
Litter incidence	N	0	4	0	6
	%	0	27	0	43
Tibia, proximal epiphysis-not ossified					
Fetal incidence	N	21	8	16	27
	%	21	8.4	15	28
Litter incidence	N	8	5	7	10
	%	53	33	47	71

Skeletal malformations (not shown in above table) that occurred in the animals included occurrence of rib and vertebral column malformations, included fused, bifurcated and absent ribs, but these effects were seen with equal frequency in controls and treated animals.

The potentially important skeletal malformations and variations seen in this study are delineated in the above table. These included an incompletely ossified hyoid body with a slightly higher incidence at the high dose (21%) versus the Controls (13%). This value was also outside the stated historical control maximum range value of 14%). Also, an increase was observed in the incidence of high-dose fetuses (21%) and litters (43%) with the middle phalanx of forelimb digit V not ossified, compared to 0% for both fetal and litter incidence in the Control animals. Furthermore, there was a slight increase in the incidence of fetuses with one or more bones of the skull not ossified (13% Control vs. 22% high dose fetal incidence) and proximal epiphysis of the tibia not ossified (21 % Control vs 27% high dose, fetal incidence). **The sponsor interpreted these data to mean that, in the high dose, the drug demonstrated a slight treatment-related retardation in fetal development, specifically, in delays in ossification of the hyoid bone and forelimb phalanges.**

Toxicokinetics

Table 20 Plasma concentrations of DHE in pregnant rabbits receiving DHE 45 Nasal Spray during Study T-2929

Dose (mgDHE base/day)	Plasma concentration of DHE (pg/ml) Day 6 pc	Day 18 pc
0.34	C_{max}* = 2,620 ± 1140 AUC_{0-24} = 13,200 ± 10,900	C_{max} = 2,749 ± 459 AUC_{0-24} = 13,900 ± 14,900
1.03	C_{max} = 4,290 ± 841 AUC_{0-24} = 23,600 ± 20,000	C_{max} = 9,090 ± 3,740 AUC_{0-24} = 22,800 ± 3,150
3.09	C_{max} = 5,020 ± 1,280 AUC_{0-24} = 60,800 ± 7,380	C_{max} = 9,140 ± 1,470 AUC_{0-24} = 63,200 ± 25,300

*t_{max} was 0.5 hour in all cases. C_{max} in pg/ml. AUC in pg.h/ml.

Absorption of the drug by the intranasal route was rapid, with a t_{max} of about 0.5 hour. Although quite variable, C_{max} and AUC were somewhat linear with dose. There appeared to be no accumulation of drug with time (6 days pc versus 18 days pc) and therefore, no time-dependent pharmacokinetics were apparent.

Dose at which effects occurred versus human dosing regimen

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. In this rabbit teratogenicity study, animals received sufficient drug at the high dose (3.6 mg/day) to attain a C_{max} of up to 9 ng/ml and AUC of up to 63 ng.h/ml. The skeletal effects found with DHE 45 Nasal Spray occurred only at this high dose, which is about a 9-fold higher C_{max} and 11-12-fold higher AUC than that resulting from clinical dosing. Therefore, there is some margin of safety with respect to these skeletal abnormalities/arrested fetal development. **However, it would be preferable if this drug were not administered to pregnant women.**

Reviewer's Comments

1. At the high dose (3.6 mg/day) there were skeletal abnormalities and variations that the sponsor interpreted to be the result of arrested fetal development. The sponsor suggested that these effects might be due to maternal toxicity, as witnessed by a slight decrease in weight (1%) from Day 6-9 pc. However, I would argue that 1) 1% loss of weight is borderline for designating maternal toxicity and 2) these effects are certainly of sufficient seriousness to prevent administration of the drug to pregnant women in this dose range. There is some margin of safety provided by the fact that this dose gave an AUC in the range of 11-12-fold higher than the AUC found with the human dosing regimen. However, the large degree of error (±50-100% in some cases) in the AUC calculations in the animal study detract considerably from that comfort level. It should be noted that the effect is not actually a teratogenic effect, so much as the result of arrested fetal development.

In the labelling (package insert) for the approved I.V. and I.M. form of DHE 45, the sponsor lists the product under Teratogenic Effects as "Pregnancy Category X", because "prolonged vasoconstriction of the uterine vessels and/or increased myometrial tone leading to reduced myometrial and placental blood flow may contribute to fetal growth retardation in animals". In the Contraindications Section the sponsor states in that labelling that "Dihydroergotamine possesses oxytocic properties and, therefore, should not be administered during pregnancy." Based on this teratogenicity study in rabbits, in which arrested fetal development in the form of **delays in ossification of the hyoid bone and forelimb phalanges** was seen, I would recommend that the DHE 45 Nasal Spray also be labelled as "Pregnancy Category X".

4. An intranasal teratology (Segment II) study in rats. GLP. Sandoz Pharmaceuticals Corp., East Hanover, New Jersey, Study #2950, June 9, 1994.

The purpose of this study was to detect possible effects on developing rat fetuses after intranasal administration of DHE 45 to pregnant on Day 6 through Day 15 of gestation at 0.16, 0.48, or 1.44 mg/rat /day (equivalent base dose levels are 0.14, 0.41 or 1.23 mg/rat/day).

Study Description

Test article

Bach #T-21018 was of DHE 45 Nasal Spray was used. The formulation consisted of a 4 mg/ml solution in Nasal Spray Placebo (previously described).

Animals

116 female rats (Charles River-CD Sprague Dawley-derived;
Animals were 13 weeks old. 25 males, 12 weeks old, were used for mating.

Dosing

Dose levels were chosen from a previously completed dose-range finding study in pregnant rats with DHE 45 Nasal Spray (Project T-2920). In the previous study , dose levels of 1.2, 1.44 and 2.0 mg/rat/day resulted in minimal maternal toxicity, as determined by decreased body weight. Doses chosen for this study were 0.16, 0.48 and 1.44 mg/rat/day (equivalent base dose levels of 0.14, 0.41 and 1.23 mg/rat/study).

Dose group	DHE 45 (mg/rat/day)	Number of 10µl droplets per nostril	Total volume administered (µl/rat/day)
Control	0	4 or 5 x daily	360
Low	0.16	2 x 1 daily	40
Mid	0.48	3 x 2 daily	120
High	1.44	4 or 5 x 4 daily	360

Observations

Clinical observations, body weights, food consumption data, fetal external exams, fetal visceral exams, and fetal skeletal exams were done. Toxicokinetic parameters were also measured. Fetal deaths and resorptions were also examined.

Results

Clinical observations and female mortality

No animals died in the study. Clinical observations were dose-related and were as follows:

Group	Control	Low	Mid	High
DHE 45 (mg/day)	0	0.16	0.48	1.44
Red salivation immediately post-dose	0	0	0	11
Salivation immediately post-dose	6	5	18	25
Gasping	0	0	1	10
Dried red substance around muzzle	0	1	3	9

No macroscopic findings were reported in mated animals at scheduled necropsy.

Maternal body weights and food consumption

A minimal tendency toward slightly lower body weight gain was suggested at the 1.44 mg/day dose level. This effect was most obvious during the early part of the study up to Day 12 pc (see following Table).

Mean percent body weight change–pregnant animals only

Group	Control	Low	Mid	High
DHE 45 (mg/day)	0	0.16	0.48	1.44
Day 0-6 Pretreatment period	11	11	10	10
Days 6-7 (Treatment period	1	1	1	1
Days 6-9	3	5	3	2
Days 6-10	4	7	5	3
Days 6-12	7	10	9	6
Days 7-12	7	8	8	5
Days 8-12	6	7	6	5
Days 12-15	5	5	5	5
Days 6-15 (Overall Treatment Period)	12	16	14	12
Days 15-20 (Post-treatment period)	25	22	24	22

Food consumption was also slightly lower (about 6%) at the high dose (1.44 mg/day) than Controls (see following Table).

Mean total absolute and relative food consumption during treatment (Days 6-15 pc).

Dose (mg/day)	0	0.16	0.48	1.44
Mean total food consumption during treatment (grams)	223	250	234	210
Relative food consumption compared to controls	100%	112%	105%	94%

Maternal Fertility and Reproductive Performance

There were no apparent effects on fertility or reproductive performance (see table below).

Table–Effects of DHE 45 Nasal Spray on fertility and reproductive performance

Group	Control	Low	Mid	High
DHE 45 (mg/day)	0	0.16	0.48	1.44
Number of animals mated	25	25	25	25
Number pregnant	21	22	24	24
Maternal mortality	0	1	0	0
Fertility index [1] (%)	84	88	96	96
Gestation index [2] (%)	100	100	100	100

[1] % matings resulting in pregnancy; includes animal 93-2611 that was electively sacrificed.
[2] % pregnancies resulting in litters with one or more viable fetuses at scheduled necropsy.

Maternal and fetal data at Cesarean section

No effect was seen on female mortality, Corpora Lutea, number dead fetuses, or number viable fetuses. However, the number of resorptions (early mainly) increased with treatment, from 5% (Control) to 14, 7.8 and 9.2% at 0.16, 0.48 and 1.44 mg/day, respectively (see Table below). This was obviously not a dose-related effect. The live fetal body weight also decreased slightly at the high dose (1.44 mg/day) (see Table below). This was reflected mainly in the female fetuses.

Maternal and fetal data at Cesarean section

Parameter	Dosage (mg/rat/day)	0	0.16	0.48	1.44
Resorptions (total and early same)	N	17	46	30	34
	%	5.2	14	7.8	9.2
	Mean	0.8	2.2	1.3	1.4
	S.D.	0.8	3.0	1.0	1.3
Live fetal body weight (g)	Mean	3.8	3.6	3.7	3.5*
	S.D.	0.5	0.3	0.2	0.2
Males	Mean	3.8	3.7	3.8	3.6
	S.D.	0.5	0.3	0.2	0.2
Females	Mean	3.7	3.5	3.6	3.4*
	S.D.	0.5	0.3	0.2	0.2

*Significantly different from Control -<0.05

Fetal Examinations

External Examinations

No treatment-related effects found.

Visceral Examinations

No treatment-related effects found.

Skeletal Examinations

Data were similar to those found in the rabbit. There were a number of skeletal variations involving incomplete ossification of bone, which is again indicative of arrested fetal development by DHE 45 (see Table 11 below). The sponsor states in the submission that "...results from skeletal examinations suggested a slight treatment-related retardation in fetal development, specifically in delays in ossification of the skull, sternum, and metacarpal V in high-dose fetus. Delays in skull development were also suggested in the low- and mid-dose groups, and reduced ossification of metacarpal V in the low-dose group." These same effects occurred in both rabbit and rat, indicating that arrested fetal development with respect to bone ossification is a consistent effect across species. These results certainly raise the concern that a similar effect will occur in the human.

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Table 11. Summary of Fetal Skeletal Variations

Parameter	Dosage (mg/rat/day)	0	0.16	0.48	1.44
Skull–one or more bones incompletely/not ossified					
Fetal incidence	N	26	60	60	65
	%	13	33	28	32
Litter incidence	N	12	18	20	21
	%	57	86	83	88
Parietal-incompletely ossified					
Fetal incidence	N	15	33	26	27
	%	7.7	18	12	13
Litter incidence	N	10	16	14	15
	%	48	76	58	63
Hyoid body–not ossified					
Fetal incidence	N	17	40	39	48
	%	8.8	22	18	24
Litter incidence	N	8	14	16	17
	%	38	67	67	71
Interparietal–incompletely ossified					
Fetal incidence	N	8	22	28	18
	%	4.1	12	13	8.8
Litter incidence	N	4	12	14	9
	%	19	57	58	38
Supraoccipital-incompletely ossifiec					
Fetal incidence	N	1	13	10	10
	%	0.5	7.2	4.7	4.9
Litter incidence	N	1	8	5	5
	%	4.8	38	21	21
Sternebra 6th-not ossified					
Fetal incidence	N	53	59	66	90
	%	27	33	31	44
Litter incidence	N	15	19	20	22
	%	71	90	83	92
Metacarpal V-not ossified					
Fetal incidence	N	75	103	92	124
	%	39	57	43	61
Litter incidence	N	17	20	51	23
	%	81	95	88	96
Total skeletal variations					
Fetal incidence	N	176	171	188	199
	%	91	94	87	98
Litter incidence	N	20	21	24	24
	%	95	100	100	100

Toxicokinetics

Table 22 Plasma concentrations of DHE in pregnant rats receiving DHE 45 Nasal Spray during Study T-2950

Dose (mgDHE base/day)	Plasma concentration of DHE (pg/ml) Day 6 pc	Day 15 pc
0.14	C_{max}* =551 ± 639 AUC_{0-24} = 3490 ± 4200	C_{max} = 1540 ± 1670 AUC_{0-24} = 11400 ± 6680
0.41	C_{max} = 2080 ± 1840 AUC_{0-24} = 19300 ± 15300	C_{max} = 4120 ± 4740 AUC_{0-24} = 30900 ± 19700
1.23	C_{max} = 10300 ± 11800 AUC_{0-24} = 56200 ± 35100	C_{max} =17000 ± 22100 AUC_{0-24} =94400 ± 63200

*t_{max} was1 hour in all cases. C_{max} in pg/ml. AUC in pg.h/ml.

Absorption of DHE 45 Nasal Spray by intranasal route was rapid, with t_{max} occurring within 1 hour of administration. AUCs are approximately linear with dose. There may be some accumulation of drug with time, as AUC values are higher on Day 15 pc than Day 6 pc. However, the large error may also explain these differences.

Dose and exposure in terms of the human dosing regimen

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 in this teratology study were exposed to doses of up to 1.23 mg/day. At this dose, animals were exposed to an AUC of up to 94.4 ng.h/ml at Day 15 pc, which is about 16-fold greater exposure than provided by the clinical dosing regimen for a given headache. With respect to C_{max}, the rat value of 17.0 ng/ml at the high dose (1.23 mg/rat/day) is about 17-fold higher than that for the human dosing regimen (1-1.3 ng/ml). However, **arrest of fetal bone ossification occurred to some extent at all doses**, and the drug should remain in "Pregnancy Category X" in the same manner as the previously approved I.V. formulation.

Conclusions

Minimal maternal toxicity was observed as slightly lower body weights at the high dose. A minimal increase (5% Controls, 7.8-14% Treated, Not dose-related) in early resorption of mainly female fetuses was found. A slight decrease in fetal body weight (8%) was also reported. The major effect was consistent with the rabbit teratology study, that of arrested fetal development in the form of delayed ossification of bone. This occurred mainly at the high dose (1.44 mg/rat/day) and could not be ruled out at the two lower doses as well. Therefore, DHE 45 Nasal Spray demonstrated reproductive toxicology effects in the form of delayed/arrested fetal development with respect to ossification of bone in the skull, sternum and metacarpal V.

Reviewer's Comments:

The arrested fetal development with respect to incomplete ossification of bone was found with DHE 45 treatment in both rabbit and rat. These results certainly raise the concern that a similar effect would be found in humans. In the labelling for the previously approved I.V. form of DHE 45, the sponsor has labelled the drug as "Pregnancy Category X" and included pregnant females in the "Contraindications" section. They did so based on the fact that "...prolonged vasoconstriction of the uterine vessels and/or increased myometrial tone leading to reduced myometrial and placental blood flow may contribute to fetal growth retardation in animals..." I recommend that DHE 45 Nasal Spray be treated in the same manner based on these reproductive toxicology results.

5. Dihydroergotamine methanesulphonate (DHE 45) fertility study in female rats, oral administration. (Project # 201-008). No GLP Statement. Batch #72007, Sandoz LTD., Basel, Switzerland, November, 1974.

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

6. Dihydroergotamine methanesulphonate (DHE-45) Fertility study in male rats (Project #201-009), oral administration. No GLP Statement. Batch 72007, Sandoz LTD, Basel, Switzerland, March, 1974.

7. Dihydroergotamine methanesulfonate (DHE 45) Perinatal and postnatal study in rats, oral administration (Project # 201-003). No GLP Statement. Volume 1.6, Batch #8612, Sandoz, LTD., Basel, Switzerland, June, 1970.

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

8. Dihydroergotamine methanesulfonate (DHE-45) Perinatal and postnatal study in rabbits, oral administration (Project 201-004). No GLP Statement. Volume 1.6, Batch #8612, Sandoz, LTD., Basel, Switzerland, December, 1973, translated from the original German document.

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

9. A teratology study in rats, oral administration (Project #201-005). No GLP Statement. Volume 1.6, Batch #8612, Sandoz, LTD., Basel, Switzerland, September, 1971.

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

10. A teratological study in rabbits, oral administration (Project #201-006). No GLP Statement. Volume 1.6, Batch #8612, Sandoz, LTD, Basel, Switzerland, September, 1971.

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

11. A teratological study in Stumptailed Macaques, oral administration (Project #201-012). No GLP Statement. Volume 1.6, Batch #72006, Sandoz, LTD, Basel, Switzerland, August, 1976.

Reviewer's Comments

These older reproductive toxicology studies (#5-11 above) were all done using oral administration and were not done under the GLP guidelines. This drug has a very low bioavailability by the oral route compared to the intranasal route (8-fold greater bioavailability by the intranasal than oral route in Cynomolgus monkeys, by both plasma and urine data), and therefore the sponsor was asked to carry out reproductive toxicology studies by the intranasal route. The sponsor complied, and those studies are reviewed as study numbers 1-4 in this Reproductive Toxicology Section of this review.

Overall Summary of Reproductive Toxicology Studies by Intranasal Route

Pregnant animals in these reproductive toxicology studies by the intranasal route were subjected to AUCs of DHE 45 Nasal Spray about 11-16-fold higher than those found with the human prescribed for the drug to treat a migraine headache. Some minimal toxicity, as evidenced by minimal weight loss, was observed in both rat and rabbit pregnant females at the high dose chosen for each species. A minimal increase in fetal resorptions and minimal decrease in fetus weights (female) were seen in rat. However, the major effect, consistently occurring in both species, was an arrested fetal development in terms of incomplete ossification of bone in skull, sternum and metacarpal V. The previously approved I.V. formulation of this drug contained labelling placing that DHE 45 formulation in the "Pregnancy Category X" due to arrested fetal development, and included "pregnant females" in the Contraindications Section. I recommend that based on the results of these more current reproductive toxicology studies in two species by the intranasal route that this DHE 45 Nasal Spray formulation be handled in the same manner.

No Segment I (fertility) or Segment III (perinatal and postnatal) reproductive toxicology have been submitted at this time. The sponsor has promised to submit such studies.

Carcinogenicity Studies

1. DHE 45 Nasal Spray carcinogenicity study in rats. Sandoz Project No. T-3038.

A protocol for a carcinogenicity study in Fischer F-344 rats was submitted to the F.D.A. in August of 1994 and was presented to the Carcinogenicity Assessment Committee (CAC). The F.D.A. sent Sandoz a letter containing the recommendations of the CAC, which Sandoz received on December 20, 1994. Sandoz in turn responded to those recommendations in an Amendment to NDA 20-148, received by the F.D.A. on January 26, 1995. During these correspondences, Sandoz has mentioned that they have begun their rat carcinogenicity study by intranasal administration. They have also been told by the CDER CAC that they may be allowed to carry out their mouse carcinogenicity study by an alternative route to the intranasal route, depending on the results of the additional genetic toxicology studies they have agreed to supply. They had requested to be allowed to use an alternative route of administration based on the difficulty they encountered administering DHE 45 Nasal Spray by the intranasal route to the mice and to the variability of the plasma level data in the mouse study.

Reviewer's Comments

For conclusions and recommendations related to the carcinogenicity studies for this NDA, see the "Summary", "Conclusions" and "Recommendations" sections of this review.

TOXICOKINETICS

1. 4-week intranasal dose range finding toxicity study in mice. GLP. Sandoz Project No. T-2876, May 9, 1994.

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

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

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. There values are not dramatically different on Day 28 versus Day 1, indicating that there is probably no accumulation of drug with time. The high variability creates difficulty in data interpretation, but there is probably no gender difference indicated for the drug in this study.

2. 13-week intra-nasal toxicity study in the Cynomolgus monkey. No GLP statement. Sandoz Project No. 203-006, May 1984.

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

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 Parent drug* (ng/ml)	Week 5 Parent drug + metabolite	Week 12 Parent drug (ng/ml)	Week 12 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 by the intranasal route. 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 probably 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%.

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

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=$AUC_{0-\infty}$ ***BQL=below quantifiable level.

Reviewer's Comments:

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.

4. 3-month intranasal maximum tolerated dose study in mice. GLP. Sandoz Study No. T-2963, August, 1994).

Toxicokinetics Studies

A satellite group of animals (6/sex/group) were used to measure plasma levels. Plasma levels were determined on Day 1 and Week 13, with bloods collected 30 minutes after the last dose in all cases. The following table shows toxicokinetics data for this study.

Plasma concentrations of DHE at designated sampling time on Week 1 (Day 1) and Week 13 of Dosing

Dose Group (mg/animal/day)	Males Plasma Conc (ng/ml) (SD)	Female Plasma Conc (ng/ml) (SD)
WEEK 1 Control	BQL*	BQL
0.04	24.8 (8.36)	16.3 (19.2)
0.12	15.3 (1.93)	35.1 (45.3)
0.16	7.1 (10.3)	23.2 (11.8)
0.2	27.3 (17.9)	19.3 (15.2)
WEEK 13 Control	BQL	BQL
0.04	18.8 (8.1)	20.2 (17.8)
0.12	25.1 (10.3)	46.2 (35.1)
0.16	35.7 (24.3)	35.2 (9.3)
0.2	35.6 (15.7)	32.6 (10.6)

BQL=Below quantifiable limit (9 ng/ml)

Reviewer's Comments:

These data demonstrate again the large variability involved in intranasal administration. Values are seen to represent up to ± 100% variability. In this study with the mouse, the plasma levels do not increase linearly with dose and on Week 13 demonstrate a plateau at about 0.12 to 0.16 mg/animal/day. There does not appear to be any accumulation of drug with time nor does there appear to be any major difference in plasma levels with animal gender. The problem of lack of linear relationship between increasing dose and plasma level may be due to the relatively small nasal cavity of the mouse. The sponsor could only administer a maximum of 5 μl liquid at a time, and with this small volume it is likely that not all of the drug remained in the nasal cavity.

Reviewer's Overall Comments Regarding Toxicokinetics

The most notable point regarding the toxicokinetics data for DHE 45 Nasal Spray, in all four toxicokinetics studies listed above for rat, mouse and Cynomolgus monkey, is the large variability with respect to plasma levels. Some of this variability is no doubt due to inherent error in the various techniques for measure of drug in the plasma. However, much of the error is most likely due to the inherent problems involved in dosing animals by the intranasal route. These problems include such simple things as the drug dripping from the nostril, the animal expelling drug by sneezing, the animal swallowing drug that inadvertently reaches the throat, and the difficulty in administering the total dose due to lack of cooperation on the part of the experimental animal. From a clinical point of view, many of these problems would, hopefully, be avoidable with human self-administration. However, with respect to the animal studies, these difficulties make it very difficult to determine accurate relationships between dose, plasma levels, and biological effects in order to evaluate the safety of the drug.

One can conclude from these toxicokinetics studies that DHE 45 Nasal Spray is absorbed from the nasal cavity with intranasal administration. Absorption is extremely variable, making analysis difficult. Also, in these studies plasma values do not necessarily represent plasma C_{max}, as bloods were collected 30 minutes after the last dose is most cases. T_{max} in rat, for example, was reported to be about 10-15 minutes after a first "puff" administration and about 2 hours after the second "puff" dose. However, it appears that in the mouse, there is some evidence that plasma concentration increases with dose, and in some cases this relationship is linear. Some evidence for linearity is also seen in the rat study, while little evidence of linearity is seen in the Cynomolgus monkey study. Mouse data provide some weak evidence (due to large variability) that there may be a plateau for absorption at the dose of 0.12 mg/day. Some evidence, although again weak, exists for such a plateau effect in rats at the 1.2 mg/day dose, especially in Week 12. Such a plateau of plasma concentration with dose could be due to a saturation of absorption. The Cynomolgus monkey data do not demonstrate such a plateau effect, but this could simply be due to differences in the surface area of the monkey nasal cavity and the fact that a saturating dose was not attained. There is no evidence for accumulation of drug with time in any of the animal studies. In the Cynomolgus monkey study, there is some evidence that plasma levels were lower at Week 12 than at Week 5. The plasma levels in this study reflect trough levels, and such an effect could be due to an increased metabolism of the drug. No obvious gender differences are apparent in the animal studies.

Summary and Evaluation

General background

Dihydroergotamine mesylate has been marketed (DHE 45®) as a sterile solution for I.V. and I.M. injections for the treatment of migraine headache. DHE is an ∝-adrenergic blocking agent and is well known for its pharmacological effects of constricting peripheral and cranial blood vessels. The compound, like other ergot alkaloids, also has the properties of serotonin agonism and antagonism. It is either the interaction of the ergots, including DHE, to various serotonin receptors, or their ability to induce vasoconstriction, that 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 permeability across the gastrointestinal mucosa and more importantly to a high first-pass hepatic metabolism. The sponsor has proposed the nasal route of administration as a way of increasing systemic availability as an alternative to parenteral administration. The nasal passages provide a highly vascularized structure for absorption (total surface are in man approximately 160 cm^2). Studies of pK and metabolism of the drug, using intranasal route in man and rat, provided the following information: 1) the drug is rapidly absorbed in a dose-dependent manner (t_{max} 15min-2hours), 2) compared to the I.V. route of administration, the bioavailability of DHE 45 is about 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 excretion, and 5) the plasma half-life is about 6-10 hours in rat and man, respectively. According to the sponsor, caffeine (10 mg) is added to the drug formulation to enhance the solubility of the drug and to act as a stabilizer. Caffeine may also increase the absorption of the drug, although it

is unclear how this might occur. Caffeine, at doses of 100-150 mg p.o., however, acts as a CNS stimulant and cerebral blood vessel vasoconstrictor (Goodman and Gilman, "The Pharmacological Basis for Therapeutics", Eighth Edition).

Pharmacology

Dihydroergotamine (DHE) is an ergot alkaloid of mixed biological activity including 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. Its efficacious effect on migraine headache is thought to be due to its vasoconstrictor activity or possibly to its interaction with the 5-HT_{1d} receptor, although the etiology of migraine headache is complex and poorly understood. Dihydroergotamine has a very low bioavailability, mainly due to an extensive first-pass metabolism. Caffeine apparently enhances the action of other ergot alkaloids, and may do the same for DHE. The major potential toxicological effects include the known role of ergot alkaloids as abortafacients and their effect on the cardiovascular system to increase blood pressure. DHE apparently has little long-term effects on blood pressure in humans, but with I.V. administration, sufficiently high plasma concentrations have been known to cause rapid increase in blood pressure of short duration (a few hours). Dihydroergotamine mesylate is already approved by the F.D.A. for intravenous administration for treatment of migraine headache.

ADME

Studies in the rat using DHE and radiolabelled-DHE dissolved into an aqueous solvent containing dextrose and caffeine in the same proportions as contained in the DHE 45 Nasal Spray examined PK by both the I.V. and intranasal routes. DHE 45 was fairly rapidly absorbed by the intranasal route, with an initial T_{max} occurring about 15 minutes after administration of the first "puff" of drug and a second T_{max} occurring at about two hours after administration of a second "puff". Depending on whether one calculates absorption of drug in terms of total radioactivity data in blood or in plasma following intranasal and I.V. dosing, or from the sum of urinary and biliary excretion data from the bile-duct cannulated rats, the fraction of the total dose absorbed was between 45-60%. Bioavailability estimates based on the ratio of AUC of plasma DHE following intranasal and I.V. dosing show bioavailability of the parent drug of about 40%. This is similar to findings in humans that showed rapid absorption of DHE after intranasal administration, with relative bioavailability of approximately 38%. With respect to drug elimination, following **I.V. administration,** the majority of the radioactivity was found in the feces (79%), with about 13% recovered in the urine. Use of bile-duct cannulated rats showed that about 81% of the 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 about 19% of the dose. **Intranasal administration** also resulted in fecal elimination as the major route (73%), compared to about 8% of the radioactivity in the urine. Use of bile-duct cannulated animals showed about 24% of the dose in the bile and 16% in the urine. 47% was found in the feces after 72 hours. Elimination of the drug by both routes of administration was rapid and essentially complete by 48 hours post-dose.

PK studies in which the spray form of DHE 45 Nasal Spray or an oral form (tablet) were administered to Cynomolgus monkeys demonstrated a $t_{1/2}$ for the drug of about 6 hours. T_{max} in these animals was about 1-2 hours, consistent with the rat. These data confirmed that the drug was fairly rapidly absorbed. These data showed that the ratio of AUC for parent drug over the AUC for parent + metabolite was 20% for the oral route and 70% for the intranasal route, indicating that more parent drug was present in plasma by the intranasal route. This was probably due to the fact that intranasal administration avoids the majority of the large hepatic first-pass metabolism seen with oral administration. The relative bioavailability of the intranasal route compared to the oral route was about

These data are important because they demonstrate that the parent drug is absorbed by the intranasal route with a bioavailability of about 40% (compared to I.V. route), indicating that the intranasal route results in superior delivery of parent drug to the plasma compared to the oral route of administration. It is known that nasal mucosa of various animal species contain a number of active cytochrome P-450 species that can be as active at metabolizing a given drug as those found in liver (Sarkar, M. Pharmaceutical Research 9(1):1-9, 92). Therefore, these data are also important in demonstrating that intranasal administration of DHE 45 results in superior delivery of parent drug to plasma, in spite of the presence of cytochrome P-450 species in the nasal mucosa. However, there is some concern about the metabolites produced by the intranasal route. It is apparent from the monkey data that some metabolism occurs, and it would be important to know the identity and nature of these metabolites, and whether or not they might be toxic, an irritant, or even carcinogenic.

Toxicology

Acute Toxicology

In one series of studies in rats and mice administered DHE by the oral and intravenous route, LD_{50} in the range of 40-100 mg/kg were seen by the oral route an in the range of 40 mg/kg for the intravenous route, yielding margins of safety of between 1300-3300-fold higher than the prescribed human dose of 2 mg (0.03 mg/kg for 60 kg person). Death of these animals was accompanied by muscle contractions, sedation and sometimes convulsions.

In a separate series of non-GLP acute toxicology studies from the 1960s involving I.V., subcutaneous, intraperitoneal and oral administration of DHE to rabbits, rats and mice, results are summarized as follows:

Species	Route of Administration	LD_{50} (mg/kg)	Margin of Safety*
MICE	I.V.	117	3900X
	Oral	none up to 2000	unknown
	Subcutaneous	none up to 625	unknown
	Intraperitoneal	212	7000X
RATS	I.V.	130	4300X
	Oral	none up to 2000	unknown
	Subcutaneous	none up to 500	unknown
	Intraperitoneal	not done	–
RABBITS	I.V.	37	1200X
	Oral	none up to 1000	unknown
	Subcutaneous	60	2000X
	Intraperitoneal	not done	–

*compared to 0.03 mg/kg dose in humans by intranasal route

Signs prior to death in these studies included drowsiness, jerking, motor excitation, forced respiration, and disturbance of equilibrium.

Data from these acute toxicology studies reveal substantial margins of safety between the prescribed human dose and the LD_{50} for DHE 45 in animals receiving the drug by the I.V., oral, subcutaneous and intraperitoneal routes of administration. **No acute toxicology data were submitted to the NDA for intranasal administration of DHE 45.** However, it is usually the case that the lowest LD_{50} for a given drug is found with I.V. administration, probably because this is the route by which the most drug arrives to the plasma (and to the sites of action) the most rapidly. Using this assumption, one would predict that the LD_{50} by the intranasal route of administration for DHE 45 would fall somewhere above the 40 mg/kg dose (the most conservative from the above studies) and provide a margin of safety of about 1000-fold with respect to LD_{50} over the prescribed human dose of 0.03 mg/kg. This also assumes that no severely toxic drug metabolite, not seen with the other routes of administration, results from metabolism by the cytochrome P-450 species in the nasal mucosa.

Subchronic Toxicology

These subchronic toxicology studies revealed that, with intranasal administration of DHE 45 to animals, toxicological effects are best broken down for discussion purposes into two categories, 1) All effects other than nasal cavity pathology and 2) nasal cavity pathology.

All Effects Other Than Nasal Cavity Pathology

In 4-week intranasal toxicology studies in rat and mouse, no deaths due to drug administration were seen. Minimal decrease in weight gain and food consumption was found in the mouse, but this was also seen in the Vehicle Control animals, indicating that the effect was probably due to the physical trauma of treatment rather than the drug. Mice and rats were exposed to plasma C_{max} levels of up to 10-fold and 25-fold, respectively, higher than levels in humans at the 2 mg dose.

Three-month intranasal mean tolerated dose (MTD) studies in rats and mice exposed animals to up to 20-fold greater plasma levels than humans receiving the prescribed dose for migraine headache. Rats demonstrated a slight decrease in food consumption at the high dose (1.6 mg/kg) accompanied by a slight weight loss. Neutrophil numbers were decreased up to 50% at the high dose and were decreased in a dose-related fashion, while total WBC counts only decreased 18% at this same dose, indicating a fairly specific effect on neutrophils. LDH levels were also elevated in the rats (HDF, 16.8%; MDM, 27%). Mice presented with decreased thyroid weights, normalized as organ:body weight ratios (HDM, 25%; HDF, 48%) and with increased LDH levels (50%, MDM; 23%, HDM).

In a 13 week intranasal toxicology study in Cynomolgus monkeys, clinical evaluation revealed an incidence of dried blood inside the nostrils and bleeding from the nose that increased in a dose-related fashion. Nasal examination of these animals revealed mucosal ulceration with scabbing or with hemorrhage. The sponsor pointed out that the ulceration and bleeding decreased in incidence with repeated dosing over the 13 week study period, in an attempt to demonstrate a lack of seriousness of this effect. However, the decreased bleeding with repeated intranasal dosing of the animals with DHE 45 could be explained by induction of constriction of the blood vessels of the nasal cavity, thus decreasing the amount of blood reaching this area. Thus, a decrease in the incidence of nasal bleeding with time might not necessarily equate with a lessening of the irritation induced by administration of the drug. With respect to drug exposure of the monkeys compared to humans receiving the prescribed dose of DHE 45, Cynomolgus monkey trough plasma levels in the study were about 1 ng/ml, while plasma C_{max} levels in humans receiving the 2 mg/headache have been reported to be about 1.1-1.3 ng/ml. Therefore, these monkeys were exposed to at least an equivalent level, and probably a higher level, of drug than one would expect for humans taking the prescribed dose.

Therefore, overall, with respect to effects other than nasal cavity pathology to be addressed in the next section of this review, there were no consistent effects with respect to classical target organ toxicity as examined in these studies, although the animals were exposed to up to 25-fold greater plasma C_{max} levels than man at the prescribed dose. The only effects worthy of note are the minimal weight loss in mouse and rat, the decreased neutrophils in 3-month rat study, increased LDH in the 3-month mouse and rat studies, decreased thyroid weights in the 3-month mouse study, and the most important effect, the nasal ulceration and bleeding in the 13-week Cynomolgus study. The increased LDH occurred in both rats and mice, but represented a minimal increases that were not dose-related. **The clinical symptoms in the monkeys are especially important, because they are consistent with the nasal cavity pathology data discussed below, and are consistent with the hypothesis that repeated intranasal administration of DHE 45 can result in chronic irritation of the nasal mucosa. Of further note and importance is the fact that nasal bleeding and ulceration occurred at all doses administered, and therefore there was no NOEL determined for this effect.**

There is also a somewhat glaring omission in the subchronic toxicology studies. The ergot alkaloids are known to affect the cardiovascular system, and DHE has apparently been shown to cause a rapid, short-lived increase in blood pressure when administered at sufficient doses by the I.V. route of administration. However, the sponsor only looked for these effects in the Cynomolgus monkey study, and then only at heart rate and ECG effects but not blood pressure. Also, it is questionable whether or not the dose was sufficient in this study to see the cardiovascular effects.

Nasal Cavity Pathology

The toxic effect of major concern with DHE 45 Nasal Spray is one that is most likely specific for the intranasal route of administration, that of chronic irritation with evidence of chronic inflammatory response in the nasal cavity. The Cynomolgus monkeys mentioned in the above section presented with nasal bleeding and ulceration of the nasal cavity with repeated intranasal administration of DHE 45 Nasal Spray over 13 weeks. Histopathology data for three animals per group revealed rhinitis (1 LDM, 1 HDM) and congestion of submucosal blood vessels (1LDM). These data indicated that DHE 45 Nasal Spray administered by repeated intranasal dosing acted as a chronic irritant. Although these data created a low level of concern with respect to the drug, the rodent data, summarized in the following table, increased the level of concern considerably.

Data summary with respect to nasal cavity histopathology results including ulceration, Goblet cell and focal respiratory epithelial cell hyperplasia and squamous metaplasia:

Four-Week Study--Rats

Note: 5 animals per group were examined.

	MALE				FEMALE			
NASAL CAVITY LEVEL	0	0.4	0.8	1.2	0	0.4	0.8	1.2
I								
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
II								
Increased prominence of goblet cells								
-minimal	0	0	0	0	0	0	0	1
-slight	0	0	0	0	0	0	2	0
I								
Focal erosion								
-minimal	0	0	0	0	0	0	0	1
-slight	0	0	0	2	0	0	0	5
II								
Focal erosion								
-minimal	0	0	0	1	0	0	0	2
-slight	0	0	0	1	0	0	0	2

Four-Week Study--Mice

Note: 5 animals per dose were examined

Organ/Parameter	Untreated Controls	Vehicle Controls	0.04	0.08	0.12
MALES					
Goblet Cell Hyperplasia					
-minimal	-	0	0	0	2
FEMALES					
Goblet Cell Hyperplasia					
-minimal	-	0	1	0	2
-slight	-	0	0	1	0
Total	-	0	1	1	2

Three-Month Study--Rats

Note: 10 animals per sex per group were examined.

Findings	Incidence of Lesions (numeric)									
	Males					Females				
	Grp 1 0 mg	Grp2 0.08	Grp4 0.72	Grp5 1.2	Grp3 1.6	Grp 1 0 mg	Grp2 0.08	Grp4 0.72	Grp5 1.2	Grp3 1.6
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
Focal squamous metaplasia										
LEVEL I:										
Very mild	0	0	0	0	0	0	1	2	0	2

Three-Month Study--Mice

Note: 10 animals per sex per group were examined.

Incidence of Lesions in the Nasal Cavity of Mice in the 3-Month Toxicology Study
Note: 10 animals per sex were examined in each dose group

Findings		Males					Females				
	Treatment Grp*	1	2	3	4	5	1	2	3	4	5
Very mild Goblet Cell Proliferation											
Level I		0	2	4	7	7	1	5	5	7	10
Very mild focal ulceration											
Level I		0	0	0	0	0	0	0	0	1	0
Mild focal ulceration											
Level III		0	0	0	1	1	0	0	0	0	0
Mild focal squamous metaplasia											
Level III		0	0	0	0	1	0	0	0	0	0

*Doses=Group 1, 0 mg/day; Group 2, 0.04 mg/day; Group 3, 0.12 mg/day; Group 4, 0.16 mg/day; Group 5, 0.20 mg/day.

Reviewer's Comments

In four-week studies, rats and mice demonstrated symptoms of chronic inflammation of the nasal cavity olfactory and respiratory epithelium such as rhinitis, eosinophilic inclusions, and of greatest concern, Goblet cell hyperplasia. 3 of 5 female and 3 of 5 male rats experienced Goblet cell hyperplasia at the high dose (0.12 mg/day, mice; 1.2 mg/day, rats). 2 of 5 female and 2 of 5 male mice showed Goblet cell hyperplasia at the high doses. This effect was surely an effect of the drug, as none of the Control animals showed similar effects. Hyperplasia is a non-neoplastic proliferative condition, often the result on chronic irritation, that can simply be a regenerative process in response to necrosis. However, it is not always clear histologically whether hyperplasia is a regenerative response or part of a morphological continuum to neoplasia. These data alone would create a mild level of concern with respect to safety of the drug administered by intranasal administration. However, the 3-month MTD studies in mice and rats presented an even more ominous finding.

With respect to the 3-month studies, the animals expressed similar histological conditions to the 4-week studies consistent with chronic inflammation. These conditions included eosinophilic inclusions in respiratory and olfactory epithelia, rhinitis, focal mucosal inflammatory cell infiltrate, and two of the mice at the high dose and one at the intermediate dose presented mild ulceration of the nasal cavity. Again, a very high incidence of the animals, 7/10 female and 7/10 male rats at the high dose (1.6 mg/kg) and 7/10 male and 10/10 female mice at the high dose (0.2 mg/kg) presented with Goblet cell hyperplasia. An effect not reported at 4 weeks, focal respiratory epithelial hyperplasia, was also present in at least 5/10 males and 5/10 females at 1.2 mg/day and 3/10 males and 5/10 females at 1.6 mg/day. Of even greater concern was the fact that female rats presented with focal squamous metaplasia (1/10, 0.08 mg/day; 2/10, 0.72 mg/day; 2/10, 1.6 mg/day) and 1/10 male mice at the high dose (0.20 mg/day) also presented with focal squamous metaplasia. Again, few, if any Control animals presented these histopathological findings, indicating that the drug was responsible for the effects.

Squamous metaplasia is a pathological condition that occurs in the respiratory epithelium in response to prolonged or continued injury. Squamous metaplasia is rarely seen in rats except in inhalation studies with irritant chemicals or with marked inflammation associated with bacterial or fungal infections. Squamous metaplasia with atypia and disorganization of the cellular layer is sometimes seen in chemically induced squamous metaplasia, and in these cases may be preneoplastic with a greater probability of progression to squamous cell carcinoma (Pathology of the Fisher Rat, E. Boorman et al., Academic Press, San Diego, 1990). The additional findings of hyperplasia of respiratory epithelia and squamous metaplasia in these 3-month studies, in addition to Goblet cell hyperplasia (also found in the 4-week studies), indicates a disturbing progression of pathology in the nasal cavity with time, apparently as a result of the chronic inflammatory condition elicited by the drug. While it is true that slightly higher doses of drug were included in the 3-month studies, these hyperplasias and metaplasias were also found at 1.2 mg/day in rat and 0.12 mg/day in mouse, the highest doses included in the 4-week studies. So an increase in drug dose cannot explain this progression of pathology with time. If this level of pathology is found in the nasal cavity of these animals after a 3-month repeat dose study, one has to wonder to what degree this pathology will have progressed with more extended exposure, such as in a two year carcinogenicity study. The presence of these findings certainly creates a high level of concern with respect to the safety of this drug administered by the intranasal route.

Another factor that increases the level of concern is the absence of a no effect level (NOEL) for a number of these histopathological findings. With respect to Goblet cell hyperplasias, in the four-week rat study, one can assign 0.4 mg/day as the NOEL. However, in the four-week mouse study and the three-month rat and mouse studies, animals presented Goblet cell hyperplasias even at the lowest doses tested, and there was therefore no NOEL detectable. The same is true for the focal respiratory epithelial hyperplasias in the nasal cavity of the rats in the three-month study. With respect to squamous metaplasias, although the incidence was low in the three-month studies, 2/10 female rats at the high dose and 1/10 male mice at the high dose, no NOEL was found for the female rats as well. Findings of these kind suggest that there may be no margin of safety for these pathological effects at

the doses of DHE 45 Nasal Spray administered to the animals in this study with respect to the human dose. It is impossible to calculate a margin of safety when no NOEL was determined.

An important factor in interpreting these data is the level of exposure of the animals to DHE 45 Nasal Spray. Since the nasal pathologies reported for this drug are most likely due to a local effect of the drug when applied directly to the nasal mucosa, the best way to compare exposure levels is probably in terms of nasal cavity surface area, or mg of drug per cm^2 surface area. This allows one to normalize exposure levels in the nasal cavities of the various animals of different sizes. The following table summarizes these exposure levels, with an eye to the lowest dose administered, since no NOEL was determined for the majority of the histopathological findings.

DHE 45 Nasal Spray Nasal Cavity Exposure Levels Expressed in Terms of Nasal Cavity Surface Area

Species	Dose	Surface area (cm^2)	Mg/cm^2 Exposure
Human	2 mg	160	0.0125
Rat (4-week study)	0.4 mg	14	0.029
Rat (3-month study)	0.08 mg	14	0.006
Mouse (4-week study)	0.04 mg	2.8	0.014
Mouse (3-month study)	0.04 mg	2.8	0.014
Monkey (13-week study)	0.5 mg	62	0.008
	1.0 mg	62	0.016
	4.0 mg	62	0.065

These doses of drug, with the exception of the monkey study, represent the lowest dose of drug administered in the specified study. Since no NOEL was determined, these dose levels also represent the lowest dose at which hyperplasias and squamous metaplasias were seen to develop in the nasal cavity of the animals. These data demonstrate that the rats and mice in these studies developed hyperplasias and female mice developed squamous metaplasias in the 3-month study at local levels of drug that were in same exposure range, or even lower exposure range, than encountered by a patient administering 2 mg of DHE 45 Nasal Spray intranasal. And I must again emphasize that these are the lowest doses used in the study, and that it remains unknown at what level these pathologies cease to occur (except for the Goblet cell hyperplasias in the 4-week rat study, with a NOEL of 0.4 mg/day).

With respect to the Cynomolgus monkeys, that exhibited clinical signs of nasal cavity irritation such as nasal bleeding and ulceration with 13 weeks of treatment with DHE 45, only minimal histopathological findings involving rhinitis and congestion of submucosal blood vessels were reported. This difference from the rodent findings cannot be explained in terms of local drug exposure level, as the monkeys clearly were exposed to drug from the same level as rodents and man to approximately six-fold higher levels. One explanation for the different pathology results for monkey and rodent is a difference in susceptibility of the animals to the irritating effects of the drug, although the high incidence of nasal bleeding among the monkeys would argue to the contrary. Another possibility is that the more serious histopathological effects in the monkeys might have been missed as the result of a sampling artifact. Monkey nasal cavity surface area of 62 cm^2 is considerably larger than 14 cm^2 for the rat or 2.8 cm^2 for the mouse, and only 3 monkeys per group were used for animal studies while 10 rodents/group were used. Therefore, the tissue sampling of a smaller surface area nasal cavity of a greater number of rodents could have considerably increased the odds of finding the pathologies in question.

Genetic Toxicology

So far the sponsor has submitted the following genetic toxicology studies:
Previously submitted:

1. Mutagenicity Evaluation using Salmonella Typhimurium (1983)
2. Micronucleus test for Mutagenic Potential in Mice (1973; non-GLP)
3. Micronucleus Test and Cytogenetic Analysis of Chinese Hamster Bone Marrow Cells for Evaluation of Mutagenic Potential (1973; non-GLP)

Submitted in Amendment dated 1/25/95:

4. Chromosome Aberration in Cells of Chinese Hamster Cell Line V79 (Doc. No. 203-127).
5. Test for the Induction of DNA Repair Synthesis (UDS) in Rat Hepatocyte Primary Cultures (Doc. No. 203-111).
6. Mutagenicity Evaluation in V79 Chinese Hamster Cells (HGPRT-Test) (Doc. No. 203-117).

Therefore, six genetic toxicology studies have been submitted to the NDA to date, three in the original submission and three in an Amendment received on 1/25/95. Two of these six studies were inadequate due to a lack of positive controls and the fact that they were not done under the GLP guidelines. The two inadequate studies were in the group submitted with the original NDA and were the following:

1. Micronucleus test for mutagenic potential in mice.
2. Micronucleus test and cytogenetic analysis of Chinese Hamster bone marrow cells for evaluation of mutagenic potential.

The sponsor has promised to send GLP studies to replace these inadequate ones. These promised studies include the following:

1. Mouse micronucleus test using current study design (due April 3, 1995).
2. Human lymphocyte assay, In Vitro test for chromosomal aberrations (due May 8, 1995).

Compliance with recommended requirements for genetic toxicology testing

When the sponsor submits results of these two additional genetic toxicology studies, they will have submitted sufficient studies to meet the recommended requirements. They will have included a bacterial gene mutation assay (Ames test), a mammalian gene cell mutation assay (chromosomal aberration assay in cells of Chinese Hamster Cell line V79), mutagenicity evaluation in V79 Chinese Hamster Cells (HGPRT-Test), human lymphocyte assay and an in vivo mutagenicity test (mouse micronucleus).

Adequacy of study results

With respect to study results, data from the chromosomal aberration test in cells of Chinese Hamster cell line V79 suggest that the drug may be clastogenic at the highest concentration (105 μg). The sponsor argues that this positive response should not be considered positive because this is the concentration of drug at which cell viability is reduced to 48%. However, while a decrease in cell viability may reduce the overall number of cells in the culture, results for number of aberrant cells at this concentration of drug was based on an analysis of 400 cells the same as with other drug concentrations and controls. It is unclear how the lower cell viability would alter the outcome of the analysis. The test for induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures indicated that no unscheduled DNA repair was induced by DHE 45. Results of the mutagenicity evaluation in V79 Chinese Hamster cells (HGPRT test) indicated no increase in mutation frequency, although a high value with the DMSO Control in the second experiment was unfortunate. The mouse

micronucleus test and micronucleus test and cytogenetic analysis of Chinese Hamster bone marrow cells for evaluation of mutagenic potential test were very old (1973) , non-GLP and did not contain sufficient numbers of animals or positive controls, and therefore were invalid studies. The mutagenicity evaluation using Salmonella typhimurium also demonstrated negative results with respect to DHE 45 at two concentrations. However, the caveat with this study was that, at the high dose in the TA1535 strain (±S9), it is impossible to determine whether or not a potentially positive response occurred due to toxicity.

In conclusion, two studies are invalid, one study indicates that DHE 45 is not mutagenic, one study demonstrates that the drug does not induce unscheduled DNA repair, one study raises the possibility of clastogenicity, and the bacterial gene mutation assay data offer a hint of positivity althought these data are problematic at the high concentration due to toxicity. **In order to render a final decision on the potential mutagenicity of the drug, we will have to review the data from the two additional studies that have been promised by the sponsor (mouse micronucleus and human lymphocyte assays). Additionally, the sponsor should submit the individual data from the bacterial gene mutation assay so the reviewer can determine whether or not the assay is sufficient as it stands or needs repeating.**

The possible positive finding in the chromosomal aberration test in Chinese Hamster V79 cells and the questionable results of the bacterial gene mutation assay are especially important in light of the findings of hyperplasias and metaplasias in the nasal cavity of rats and mice in the 3-week and 4-month animal toxicology studies. While by no means conclusive evidence for carcinogenic potential for the drug in absence of the results of the actual carcinogenicity studies in rodents, these potentially positive genetic toxicology findings in addition to the histopathological findings in rodents again raises the level of concern for the safety of this drug by this route of administration. The results of the additional two genetic toxicology studies promised by the sponsor will be very important in determining whether or not the drug is clastogenic or mutagenic.

Reproductive Toxicology

Pregnant animals in these reproductive toxicology studies in rats and rabbits by the intranasal route were subjected to AUCs of DHE 45 Nasal Spray about 11-16-fold higher than those found with the human prescribed for the drug to treat a migraine headache. Some minimal toxicity, as evidenced by minimal weight loss, was observed in both rat and rabbit pregnant females at the high dose chosen for each species. A minimal increase in fetal resorptions and minimal decrease in fetus weights (female) were seen in rat. Although ergot alkaloids in general are known to act as abortifacients, no increase in fetal abortion was seen in these studies. The major effect, consistently occurring in both species, was an arrested fetal development in terms of incomplete ossification of bone in skull, sternum and metacarpal V.

The previously approved I.V. formulation of this drug contained labelling placing that DHE 45 formulation in the "Pregnancy Category X" due to arrested fetal development, and included "pregnant females" in the Contraindications Section. I recommend that based on the results of these more current reproductive toxicology studies in two species by the intranasal route that this DHE 45 Nasal Spray formulation be handled in the same manner. Additionally, considering the potential for overuse of DHE 45 Nasal Spray in terms of its ease of administration and the fact that higher doses of the drug may, in fact, stimulate uterine contraction, "Pregnancy Category X" seems an even better idea.

Carcinogenicity Studies

A carcinogenicity study including administration of DHE 45 Nasal Spray to Fischer F-344 rats by the intranasal route has recently been started by the sponsor. The sponsor has requested a waiver for the mouse carcinogenicity study by the intranasal route based on the difficulties with administration of the drug intranasal to the mice and the resultant large variability of plasma levels in the animals receiving the same dose of drug. F.D.A. and the sponsor then discussed the possibility of carrying out the carcinogenicity study in mice by an alternative route of administration, and the sponsor expressed a desire to have the carcinogenicity study in mice waived completely. F.D.A. responded to this request in a letter to Sandoz on December 20, 1994, in which the F.D.A. stated the following:

"Before the Agency may consider waiving the carcinogenicity study in mice by an alternative route, you (the sponsor) must provide a basic core battery of genetic toxicity studies by current standards. As part of your NDA submission you have provided a mutagenicity screen consisting of the following three tests: a) Mutagenicity Evaluation Using Salmonella Typhimurium (1983), b) Micronucleus Test for Mutagenic Potential in Mice (1973) and c) Micronucleus Test and Cytogenetic Analysis of Chinese Hamster Bone Marrow Cells for Evaluation of Mutagenic Potential (1973). Studies b and c were not GLP studies. In order to complete the required basic mutagenicity screen by 1994 standards you must submit both an in vitro chromosomal aberrations test and an in vivo chromosomal aberrations test with Good Laboratory Practices regulations."

At present, the sponsor still has to submit the results from the following two additional genetic toxicology studies: 1) Mouse micronucleus test using current study design (due April 3, 1995) and 2) Human lymphocyte assay, In Vitro test for chromosomal aberrations (due May 8, 1995). Therefore, a final conclusion as to the potential mutagenicity of DHE 45 Nasal Spray has yet to be determined and the F.D.A. is not yet in a position to decide on the requirement for a mouse carcinogenicity study. However, based on the fact that 1) the CDER Carcinogenicity Assessment Committee recommended, and the sponsor agreed, to decrease the doses for the carcinogenicity study in rats, thus effectively reducing the systemic exposure of potential target organ systems to the drug, and 2) the fact that there are no 6-month or one year toxicology studies available for this drug, I recommend that a carcinogenicity study be carried out in the mouse by an alternative route of administration to the intranasal route. The carcinogenicity study in the rat by the intranasal route should answer questions relating to the worrisome histopathology findings in the nasal cavity and their relevance to the possible development of neoplasias. The purpose of a carcinogenicity study in mice by an alternative route is to ensure that the issue of carcinogenicity in target organs other than the nasal cavity unique to intranasal administration be addressed at an appropriate exposure level relative to the prescribed dose of 2 mg for humans.

Toxicokinetics

The most notable point regarding the toxicokinetics data for DHE 45 Nasal Spray, in all four toxicokinetics studies listed above for rat, mouse and Cynomolgus monkey, is the large variability with respect to plasma levels. With respect to the animal studies, this large variability and a lack of ability to determine NOELs with respect to some of the histopathology findings, makes it difficult to determine accurate relationships between dose, plasma levels, and biological effects and to determine a margin of safety for the drug. There is no evidence for accumulation of the drug with time in any of the animal studies. In the Cynomolgus monkey studies, there is some evidence that the plasma levels were lower at Week 12 than Week 5, which could be explained by increased metabolism of the drug. No obvious gender differences were apparent in the animal studies with respect to toxicokinetics.

CONCLUSIONS

Pharmacology

The pharmacology of DHE 45 Nasal Spray is consistent with the treatment of migraine, and the drug is already approved for this indication by the intravenous (I.V.) and intramuscular (I.M.) route of administration. PK and toxicokinetics studies indicate that the drug is adequately absorbed by the intranasal route, with a bioavailability in rats and man of about 40% versus I.V. administration. Large variability of the plasma level values was of concern from the perspective of preclinical data interpretation and actual exposure levels in the animals. There were no toxicokinetic effects with respect to drug accumulation or gender differences that would raise concern. 13-week Cynomolgus monkey studies using radiolabelled drug revealed that, with intranasal administration, there was still considerable metabolism of the drug. One concern is that there is no information provided as to the identity or nature of the metabolites. It is known that there are active cytochrome P-450 species in the nasal mucosa, and it is of some concern that metabolism of the drug at this site may result in a metabolite that is potentially toxic, an irritant, or even carcinogenic. In the absence of data, it is impossible to reach any conclusion in this matter.

Acute Toxicology

Acute toxicology data by the intravenous, oral, subcutaneous and intraperitoneal route in a number of different animal species revealed satisfactory margins of safety for DHE 45 with respect to LD_{50}.

Subchronic Toxicology

For a drug with a chronic intermittent indication such as DHE 45® Nasal Spray for migraine headache, the normal requlatory requirements include a six month study in rodents and a one year study in a non-rodent species, by the same route of administration as that planned for the clinic. The studies of longest duration utilizing the intranasal route of administration submitted to this NDA to date are the 3-month maximum tolerated dose (MTD) studies in rats and mice and the 13-week study in Cynomolgus monkeys. Very old studies (from 1969) including 26-week non-GLP studies in rats and dogs by the oral route of administration were included by the sponsor, but the age of the studies, the route of administration (oral) different from that prescribed for the clinic (intranasal), and the known very poor bioavailability of the drug by the oral route render them of little use in determining the safety of DHE 45® Nasal Spray. Therefore, the sponsor has not provided the minimally required number of studies of appropriate duration and route of administration to support this type of submission. This is of even greater concern because the histopathology findings in the nasal cavity of rats and mice, discussed in more detail below, appeared to become progressively worse with time.

In a letter from the F.D.A. to the sponsor on December 20, 1994, addressing the recommendations of the CDER Carcinogenicity Assessment Committee, the following requests were made in an attempt to at least partially rectify this situation:

> **FDA Recommendation**: " The carcinogenicity study will also serve as a chronic toxicity study; therefore you (the sponsor) should include satellite animals (a minimum of 5/sex/group) for the purpose of assessing plasma drug levels and clinical pathology parameters. Sampling and analysis should be performed at least twice during the study. Based on the difficulties with blood sampling from the tail vein demonstrated in the dose ranging study, an alternative strategy should be considered."

The sponsor answered this request in an Amendment to NDA 20-148, received by the F.D.A. on 1/26/95, with the following response:

> **Sandoz Response**: "This study was not designed nor was it intended to serve as a combination chronic toxicity/carcinogenicity study. Chronic toxicity studies included in the NDA were 26-week rat and dog oral studies (Doc. Nos. 201-001 and 201-002). However, the Sponsor will agree to designate 5 rats/sex/group for clinical pathology bleeds at 6 and 12 month time points in this study. Plasma drug levels are already included in the study design and will be addressed in a later question. We believe that blood sampling from the tail vein is the only viable alternative in this study, since bleeding from the retro-orbital plexus carries the risk of possible sample contamination from drug residues which may be present on the nose or snout."

The sponsor also agreed in this Amendment to additional data collection in this study, including assessment of organ weights at terminal sacrifice and histopathological examination of a number of tissues, including the respiratory tissues, in the Control, Low- and High-dose groups. Therefore, the sponsor has agreed to provide some additional data to partially satisfy the requirements for chronic (1-year) toxicology studies. However, those data will not be received by the F.D.A. until the two year carcinogenicity studies are completed.

With respect to the results of the intranasal subchronic toxicology studies that were provided, the findings of greatest concern were the histopathological effects of intranasal administration of DHE 45 Nasal Spray on the nasal cavity of rats and mice. These data from 4-week and 3-month studies are characterized in detail in the "Summary" Section of this review under the heading of "Subchronic Toxicology" and the subheading of "Nasal Cavity Pathology". These data show that repeated intranasal administration of this drug results in a chronic inflammatory response in the nasal cavity. The pathology progressed with time, from the presence of indicators of inflammation (eosinophilic inclusions, rhinitis) and Goblet cell hyperplasia in the 4-week studies to a higher incidence of Goblet cell hyperplasia and the appearance of hyperplasia of the respiratory epithelium and squamous metaplasia at three months. Hyperplasias and metaplasias, as described in the "Summary" section, can be pre-neoplastic conditions. Also of importance is the fact that in the majority of the cases, no apparent no-effect-level (NOEL) was detectable for the hyperplasias and squamous metaplasia. In the 13-week intranasal Cynomolgus monkey study, animals demonstrated nasal bleeding and ulceration of the nasal cavity, but histopathology included mainly focal rhinitis. However, one concern with this study is that with the smaller numbers of monkeys in the studies (3/group) and the sampling of tissue from the larger surface area of the nasal cavity in monkeys (62 cm^2 versus 14 cm^2 for rat and 2.8 cm^2 for mouse), the more serious histopathologies may have been missed.

Although ergot alkaloids are known to affect the cardiovascular system, there were no effects on electrocardiograms (ECG) or heart rates in the 13-week Cynomolgus monkey study. There was no evidence that blood pressure was monitored. These animals were only exposed to comparable plasma levels of drug to levels found in patients administered the prescribed dose of DHE 45 Nasal Spray, so there was no effort to push the dose to determine a margin of safety. No other studies were submitted with the NDA in which effects of the drug by intranasal administration on the cardiovascular system were evaluated, and this comprises a weakness in the submission.

Other than the nasal cavity histopathology effects, there were no other toxicological findings that were consistent between species and that might raise the level of concern for the safety of this drug. Decreased neutrophil numbers and decreased thyroid weights occurred, but each only appeared in a single study and a single species. Increased LDH levels occurred in both mouse and rat, but the increases were minimal. Only the Cynomolgus monkey nasal bleeding and ulceration was of major concern, because it is consistent with the general theme that this drug causes chronic irritation and a chronic inflammatory response in the nasal cavity when administered by the intranasal route. The

biological significance of this effect is emphasized in the histopathology results in the 4-week and 3-month toxicology studies in rats and mice.

The overall conclusion with respect to the subchronic toxicology studies is that the sponsor has not provided animal studies of sufficient duration and appropriate route of administration to support a submission for a drug for chronic, intermittent administration. Some attempt is being made to rectify this condition through modification of the carcinogenicity studies to include additional data normally provided from 1 year toxicology studies. However, these data will not be available until the completion of the carcinogenicity studies. Furthermore, the histopathology findings in the nasal cavities of rats and mice from 4-week and 3-month intranasal toxicology studies demonstrated hyperplasias and squamous metaplasias, conditions that raise the specter of potential carcinogenicity and for which there was no NOEL determined. These findings lead me to the conclusion that the safety of this drug cannot be adequately evaluated in the absence of the results of the carcinogenicity studies. These studies are crucial both to evaluate the carcinogenic potential in the nasal cavity already implicated in the subchronic studies in rodents and from the perspective of providing data to at least partially meet the requirements for long-term studies (6 month rodent, 1 year non-rodent) usually required for an NDA submission of this type.

Mutagenicity Studies

Requirements for examining the potential mutagenicity of a drug in an NDA include a standard battery of tests composed of *in vitro* tests including a bacterial gene mutation assay, a mammalian gene cell mutation assay, and a chromosomal aberration test and *in vivo* tests including a mouse test to look for chromosamal aberrations, such as the mouse bone marrow test. To date, the sponsor has not met these requirements. The sponsor has acknowledged this, and has promised to submit results of the following studies to complete the battery sometime in 1995:

1. Mouse micronucleus test using current study design (due April 3, 1995).
2. Human lymphocyte assay, In Vitro test for chromosomal aberrations (due May 8, 1995).

With respect to study results received to date, data from a chromosomal aberration test in cells of Chinese Hamster cell line V79 suggest at the highest concentration utilized (105 μg) that the drug may be clastogenic. The test for induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures indicated that no unscheduled DNA repair was induced by DHE 45. Results of the mutagenicity evaluation in V79 Chinese Hamster cells (HGPRT test) indicated no increase in mutation frequency, although a high value with the DMSO Control in the second experiment was unfortunate. The mouse micronucleus test and micronucleus test and cytogenetic analysis of Chinese Hamster bone marrow cells for evaluation of mutagenic potential test were very old (1973) , non-GLP and did not contain sufficient numbers of animals or positive controls, and therefore were invalid studies. In the mutagenicity evaluation using Salmonella typhimurium, the sponsor did not submit data for sufficient concentrations of drug to allow an adequate analysis for mutagenicity. Of the three concentrations tested, two demonstrated negative results with respect to DHE 45, while at the high dose in the TA1535 strain ($\pm$S9), it is impossible to determine whether or not a potentially positive response occurred due to toxicity. In order to render a final decision on the potential mutagenicity of the drug, we will have to review the data from the two additional studies that have been promised by the sponsor (mouse micronucleus and human lymphocyte assays) for sometime in 1995. The hint of positivity for mutagenicity in the data already submitted raises the level of concern for approval of this drug without the results of the carcinogenicity studies to an even higher level.

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RECOMMENDATIONS

I.

1. Based solely on the preclinical data submitted by the sponsor to this NDA to date, I must conclude that the NDA is not approvable at this time. It is this reviewer's assumption that due to ease of self-administration of the drug by the intranasal route, approval for the intranasal route of administration will result in exposure of a much larger patient population to the drug than the previously approved I.V. or I.M. routes of administration for the same indication. It is also this reviewer's assumption that for the treatment of migraine, intranasal DHE 45 Nasal Spray will be administered repeatedly, potentially for long periods of time in patients who suffer chronically from migraine headaches. Normally, for approval of an NDA submitted for chronic intermittent use, we require a one year toxicology study in a non-rodent species and a six month study in rodents plus carcinogenicity studies in rat and mouse. In this NDA submission, the longest toxicology studies are four-month studies in rat and mouse,

complete evaluation of the safety of this drug. Therefore, this NDA is not approvable at this time.

2. I recommend, for the reasons stated in the "Conclusions" section of this review, that the sponsor be required to

3. I recommend that DHE 45® Nasal Spray be labelled "Pregnancy Category X", as are the previously approved I.V. and I.M. formulations of dihydroergotamine mesylate. This is based on the reproductive toxicology findings of arrested ossification of bone and the potential, as with other ergot alkaloids, for the drug at sufficient doses (as might be attained with overuse) to act as an abortifacient.

4. I recommend that the outstanding preclinical studies, including the studies, be completed and submitted to the F.D.A.

Under the conditions of immediate approval based on clinical considerations, I would also highly recommend that the outstanding preclinical studies, especially studies (both rats and mice as previously discussed) be completed as soon as possible and submitted to the F.D.A. in case the inferences drawn from histopathology findings in the nasal cavity of animals in the subchronic toxicology studies by the intranasal route with respect to carcinogenicity are correct.

LABELLING

The latest draft of labelling (package insert) for DHE 45® Nasal Spray was submitted to HFD-120 on May 29, 1992. My recommendations regarding labelling for this NDA #20-148 are based on this labelling, with relevant comments also incorporated from the latest draft labelling of the I.V. formulation submitted to HFD-120 on August 18, 1994.

Comments and Recommendations Regarding Labelling
Note: Newly added text is bolded.

1. The chemical formula on the first page is upside down.

2. In the "Mechanism of Action" Section, a [1] should be added as follows: "This stimulating of the serotonin effect...loss of tone of the extracranial vascular musculature.[1] " This sentence should be referenced, as it is in the labelling for the I.V. formulation, with the following literature source:

Peroutka, Stephen J., M.D., Ph.D. The Pharmacology of current Anti-Migraine Drugs. *Headache*. January 1990, ppg. 5-11.

3. In the "Pharmacokinetics and Metabolism" section, the labelling for the already approved I.V./I.M. formulation of DHE contained the following sections that should also be included in the DHE 45 Nasal Spray labelling:

a) Metabolism: At least four DHE metabolites have been identified both *in vivo* and *in vitro* in preparations of human microsomes. The main metabolite is 8'ß-hydroxy-dihydroergotamine (8'-OH-DHE). 8'-OH-DHE appears to be equipotent to DHE in a number of receptor binding studies and in several venoconstrictor activity models, both *in vivo* and *in vitro*. 8'-OH DHE is further metabolized by an additional hydroxylation at the 10' position.

b. Population: Gender and age do not appear to have an impact on DHE pharmacokinetics. The use of DHE is contraindicated in persons with severely impaired hepatic function.

c. Interactions: DHE metabolism appears to be inhibited by macrolide antibiotics such as erythromycin. The pathway(s) of metabolism inhibited by the macrolides have not been determined. The concomittant use of oral contraceptives by female patients does not appear to influence the disposition of DHE.

4. In the "Indications and Usage" section, the wording "DHE 45 Nasal Spray is indicated for the symptomatic treatment of common or classic migraine headaches..." could be misinterpreted to mean treatment of common headaches or classic migraine headaches. However, the drug is only to be prescribed for migraine headache (see "Precaution" Section of labelling). This sentence should be reworded to say **"DHE 45 Nasal Spray is indicated tor the symptomatic treatment of migraine headaches, both common and classic, ...".**

5. In the "Contraindications" section of the approved I.V. drug, the work "shock" is included, and should be included in the nasal spray labelling, as follows:

"...The drug is also contraindicated in patients having conditions predisposing to vasospastic reactions such as known peripheral arterial disease, coronary artery disease (in particular, unstable or vasospastic angina), sepsis, **shock**, vascular surgery,..."

6. In the "PRECAUTIONS" section, under "Information for Patients", the first paragraph should be modified to read as follows:

DHE 45 Nasal Spray should be used only for vascular headaches of the migraine type. It has not been evaluated for other types of headaches. No more than four sprays (2.0 mg) of DHE 45® Nasal Spray should be administered during any 24 hour period...

7. The "Carcinogenesis, Mutagenesis, Impairment of Fertility" section should read as follows:

No long-term studies in animals have been completed to date to evaluate carcinogenic potential, so no conclusions regarding the carcinogenicity of DHE 45® Nasal Spray are possible. Long-term carcinogenicity studies in rodents by the intranasal route of administration are ongoing. However, preclinical data in subchronic (4-week and 3-month) toxicology studies by the intranasal route in which rodents received drug at exposure levels of 25-30-fold higher than humans receiving the prescribed dose of 2 mg per headache demonstrated the presence of a chronic inflammation in the nasal cavity that apparently progressed with repeated dosing over time to the development of Goblet cell and respiratory epithelial cell hyperplasias and squamous metaplasias. Although classified as non-neoplastic lesions, these conditions can represent part of a morphological continuum to neoplasia. In other studies, rats fed 59% crude ergot (unpurified dihydroergotamine mesylate) for up to 2 years developed tumors on the ears.

The mutagenicity of DHE 45® Nasal Spray has not yet been adequately determined. To date one study (a Chromosomal Aberration Test in Cells of Chinese Hamster Cell Line V79)

gave a positive result at the highest concentration utilized, data from a second test (Mutagenicity Evaluation Using the Bacterial Strain Salmonella typhimurium) were uninterpretable at the highest dose and an insufficient number of doses were presented, two tests (Test for the Induction of DNA Repair Synthesis in Rat Hepatocyte Primary Cultures and Mutagenicity Evaluation in V79 Chinese Hamster Cells, HGPRT-Test) gave negative results, and two studies were inadequate for evaluation. Additional tests to complete a full battery of carcinogenicity testing and to allow a final determination on the mutagenicity of DHE 45® Nasal Spray have not yet been submitted.

Segment I (fertility and reproductive performance) and Segment III (perinatal and postnatal) reproductive toxicology studies have not been completed, so no conclusions regarding this drug can be made with respect to effects on fertility, reproductive performance or effects on perinatal or postnatal health.

8. The "Teratogenic Effects" section should read as follows:

Pregnancy Category X: It is not known whether DHE 45® Nasal Spray can cause fetal harm when administered to pregnant women, or can affect reproduction capacity. However, prolonged vasoconstriction of the uterine vessels and/or increased myometrial tone leading to reduced myometrial and placental blood flow may contribute to fetal growth retardation in animals. In Segment II (teratology) reproductive toxicology studies in which rabbits or rats were administered DHE 45® Nasal Spray by the intranasal route, at doses resulting in exposure levels 11-12-fold higher than those in humans receiving the prescribed dose of 2 mg per headache, arrested fetal development in the form of incomplete ossification of bone in skull, sternum and metacarpal V was seen in the rabbits. A no effect level (NOEL) was seen in the rabbits at a dose resulting in 4-fold higher exposure levels than the human dose. In the rat study these same symptoms of arrested fetal development were found, and no NOEL was apparent, with effects occuring at exposure levels as low as 2-fold higher than the human levels.

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

CC
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HFD-120
/G.Fitzgerald/J.J.Jessop/R. Nighswander