FEB 2 1996

# REVIEW FOR HFD-120
# OFFICE OF NEW DRUG CHEMISTRY
# MICROBIOLOGY STAFF HFD-805

RETURN

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## Microbiologist's Review # 1 of NDA 20-626
## January 30, 1996

A. 1. APPLICATION NUMBER: 20-626

APPLICANT: Glaxo

2. PRODUCT NAMES: Imitrex (Sumatriptan) Nasal Spray

3. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Single dose. 5 mg, 10 mg and 20 mg of sumatriptan in a 100 ul dose volume for topical administration to the nasal mucosa.

4. METHOD(S) OF STERILIZATION: The drug is labeled non-sterile.

5. PHARMACOLOGICAL CATEGORY: Indicated for acute treatment of migraine.

B. 1. DATE OF INITIAL SUBMISSION: August 29,1995

2. AMENDMENT: none

3. RELATED DOCUMENTS: none

4. ASSIGNED FOR REVIEW: January 22,1996

C. REMARKS:

The bulk drug solution is filled followed by sterilization. The vial containing the drug is then inserted into a non-sterile nasal spray device. Since the dose volume is very small (100ul), endotoxin is not an issue and is therefore not reviewed here. The subject drug is single dose and preservative-free. The drug is to be manufactured at Glaxo in Parma, Italy.

D. CONCLUSIONS:

The data submitted on microbial limit tests and device integrity are satisfactory. The submission is recommended for approval with respect to microbiology.

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Brenda Uratani 1/30/96
Brenda Uratani, Ph.D.
Date 2/2/96

cc:

NDA 20-626
HFD 120/Div. File
HFD 805 /Uratani
HFD 120/Grilley
HFD 120/Bates
drafted by: Brenda Uratani, 1/30//96
R/D initialed by P.Cooney, 1/30/96

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# CENTER FOR DRUG EVALUATION AND RESEARCH

## APPLICATION NUMBER: 20626

## PHARMACOLOGY REVIEW(S)

# PHARMACOLOGIST'S REVIEW OF RESPONSE TO NOT APPROVABLE LETTER FOR NDA 20-626

Reviewer: Andrea M. Powell, Ph.D.

Date of Review: 2/24/97

Sponsor: Glaxo Wellcome Inc.
Research Triangle Park, NC 27709

Drug: Sumatriptan, Imitrex™ Nasal Spray

Code Number: Nasal Spray

Indication: acute treatment of migraine headaches

Dosage Form: nasal spray (5 mg, 10 mg and 20 mg)

Clinical Dose: proposed single dose of 20 mg (10 and 5 mg will be available)
maximum daily dose of 40 mg

Note: Portions of this review were excerpted directly from the sponsor's submission and the consultants' reviews.

Administrative History:

This NDA for intranasal Imitrex was reviewed and determined to be not approvable and the not approvable letter was issued on 8/28/96. The sponsor replied to this with a three volume submission (correspondence date 10/29/96, CDER stamp date 10/30/96 and received by reviewer 11/6/96). In this submission the sponsor states: "This submission represents a full response to the Agency's comments and provides additional preclinical and clinical data to support the safety of long term use of Imitrex Nasal Spray." A brief review of the information presented suggested the need for a veterinary pathology consultation on behalf of the Agency to provide an independent assessment of the apparent treatment related toxicity and irritation to nasal and respiratory systems demonstrated in the subchronic preclinical toxicity studies (see Memorandum to File for NDA 20-626, dated 12/12/96). An arrangement was made with LuAnn McKinney, DVM, DVACP, LTC, US Army from the Registry of Toxicologic Pathology at AFIP on 1/6/97 to serve as our consultant. On 1/6/97 the Agency faxed a request to the sponsor to supply the nasal and respiratory slides from the preclinical studies in a blinded fashion for consultation. On 1/24/97 the sponsor delivered the slides and the necessary documentation for the blinding and coding. On 1/28/97 the reviewing pharmacologist and Dr. Fitzgerald, the Pharmacology Team Leader met with Dr. McKinney and her colleague Dr. Tim O'Neil, also from AFIP, to go over the details of the consultation. Due to the Division's time constraints, the full consultation, as originally planned, could not be carried out. An abbreviated format for the consultation was agreed to, and is further explained, later in the review.

The preclinical issues which ultimately contributed to the not approvable status of this NDA and were delineated in the letter to the sponsor were as follows:

> "Preclinical data have raised concerns about the toxicity associated with chronic exposure and carcinogenic potential of this treatment. The results of subchronic testing with Imitrex indicate that squamous metaplasia occurred in the nasal and laryngeal epithelium of rats after 3, 8 or 35 days of inhalation treatment with a preserved formulation and in the bronchial epithelium of one dog after 14 days of intranasal treatment with the clinical formulation. Although no metaplasia were reported in dogs treated intranasally with other formulations for 13 weeks, other pathologies of respiratory tissues, which are difficult to interpret given the information which was submitted, were reported.
>
> We are concerned about these findings for several reasons:
>
> 1. The interpretation of the significance of the findings of metaplasia is impossible without a more detailed report of the pathology. For example, it would be important to distinguish between adaptive squamous metaplasia and squamous metaplasia with prominent keratinization in order to assess the potential progression to neoplasia. It is also important to determine the type of epithelium in the larynx which is being affected, squamous or respiratory, since the latter may be more indicative of metaplastic changes.
>
> 2. Although the pathology reported in the 35-day rat inhalation study appears to be reversible after a 2-week period, there is no assurance that this would be the case after chronic (at least 6 months) treatment. There is also no assurance that the pathology would not have become more serious or widespread with longer exposure. Although the use of Imitrex is intermittent, it is used repeatedly over long periods, and as such it is considered important to evaluate the potential risks associated with chronic use.
>
> 3. The estimated exposures (doses per nasal surface area in $mg/cm^2$) in rats with pathology were only equal to or twice the estimated exposure in humans receiving the maximum total daily dose. The margin of exposure in dogs is two to four times human exposure. The fact that there is virtually no safety margin for the observed pathology heightens our concern.
>
> These preclinical issues should be addressed. If it can be

determined that there is no toxicity suggestive of proliferative or pre-neoplastic changes observed as a consequence of intranasal Imitrex administration, the oral carcinogenicity studies already conducted may support the intranasal dosage form. If this cannot be shown, it may be necessary for you to conduct a 6-month intranasal toxicity study with a reversibility phase in the most appropriate species in order to determine whether or not the squamous metaplasia observed in the subacute studies progresses in severity over time. Our staff would be happy to work with you in order to address the concerns the submitted studies have raised."



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The Sponsor's Response to the Preclinical Issues from the Not Approvable Letter:

In their response to the Agency's not approvable comments the sponsor has supplied further histopathology information about the results of their previously submitted preclinical studies and has concluded: "The information provided supports the conclusion that the pathology observations in the Imitrex Nasal Spray preclinical studies are reflective of adaptive changes rather than proliferative or pre-neoplastic changes and that the most appropriate animal species for this route of administration is the dog rather than the rat." This further interpretation was based, at least in part, on an independent pathology consultation, which the sponsor had carried out, and the report of which was supplied in this submission. This consultation was carried out by

In the sponsor's reanalysis of the histopathology data from the preclinical studies the sponsor summarizes and evaluates the findings as follows:

With reference to the inhalation studies in the rat:

1. With reference to significant findings in the nasal cavity, the sponsor notes that a single rat in the eight day pilot inhalation study (# R12285) had notations of squamous metaplasia "only at a high dose (91.5 mg/kg/day administered over 120 minutes..." The sponsor states: "Given that this was a single occurrence at a very high dose in the rat, we did not consider this finding of significance in assessing the safety of sumatriptan."

2. With reference to the treatment related findings in the rat larynx, the sponsor discussed the following issues:
   a. There is no analogous structure to the rat's laryngeal ventral cartilage in humans and therefore the treatment related necrosis was "unlikely to be of any toxicological relevance to man."

   b. The treatment related changes in the epithelium of the rat larynx consisted of:
      - hyperplasia of the ventrolateral epithelium (normally cuboidal) with occasional squamous metaplasia
      - hyperplasia of the ventral epithelium (normally squamous) with occasional keratinization
      - hyperplasia of lateral epithelium (normally squamous)
      - hyperplasia of medial (inner) surfaces of arytenoid processes (normally squamous) with occasional keratinization

      The sponsor further summarized that: "Keratinization was confined to the hyperplastic squamous epithelium and squamous metaplasia confined to the cuboidal epithelium; the respiratory epithelium of the larynx (pseudostratified ciliated columnar epithelium) was not affected. Squamous metaplasia with prominent keratinization was not observed in

any inhalation or intranasal study with sumatriptan."

The sponsor contends that these histopathologic changes are adaptive responses to the inhaled formulation. They further state: "The histological appearance of these findings was of a benign and orderly nature; there was no evidence of any cellular atypia or disorganization which could indicate pre-neoplasia." The sponsor suggests that the degree of recovery demonstrated after 14 days with no treatment further supports the contention: "...that the response observed in both the squamous and cuboidal epithelia of the larynx represents an adaptive defense mechanism where a susceptible epithelium is replaced by a more resistant type." The sponsor also states that the lack of a significant treatment related histopathology in the 14 day intranasal study in cynomolgus monkeys and 91 day intranasal study in dogs "suggests that the rat larynx over-predicts as a model for human respiratory tract irritancy, and that the epithelial changes in the rat larynx are of no clinical significance."

3. With reference to the histopathological findings in the intranasal toxicity studies in dogs the sponsor noted the following and concluded that there were no significant findings.

   a. The squamous metaplasia of the bronchial epithelium in one dog administered 120 mg/dog/day of the buffered clinical formulation should not be considered a significant treatment related finding because it occurred in only one dog in one study, and, a control animal from a Glaxo Wellcome study carried out at Huntingdon Research Center has also demonstrated this finding.

   b. The epithelial erosion in the maxilloturbinates in one dog administered 480 mg/dog/day of the unbuffered, sweetened formulation for 13 weeks should not be considered a significant treatment related finding because it occurred in only one dog in one study, and, has occurred in the vehicle control animals of other studies conducted by Glaxo Wellcome.

   c. The focal epithelial hyperplasia in one dog administered 480 mg/dog/day of the unbuffered, sweetened formulation for 13 weeks should not be considered a significant treatment related finding because it occurred in only one dog in one study, and, has occurred in the vehicle control animals of other studies conducted by Glaxo Wellcome.

   d. The fibrosing alveolitis demonstrated in the 13 week study with the sweetened formulation should not be considered a significant treatment related finding because it occurred only in females, a treatment related increase was not observed in other studies, and, "All of the remaining features of the lung pathology were of comparable incidence and severity in treated and control groups and, thus, did not exhibit any treatment related effects..."

As mentioned above, the sponsor's further interpretation of the histopathology data was based, at least in part, on an independent pathology consultation, which was carried out by

Illustrative quotations from his review are presented in the following discussions; however, the full text from his consultation is appended to provide contextual reference (appendix 1).

In his executive summary states that the objectives of his review were: "(a) to assess the quality of the histological materials, (b) to assess the interpretation of the lesions by the original study pathologists, (c) to interpret the relationship to treatment of responses in the respiratory tract, and (d) to address the potential neoplastic or preneoplastic nature of any treatment-related changes observed."

In answer to issue (a) he states: "Histological materials from both rats and dogs were of high quality and were suitable for the study objectives." In answer to issue (b) he states: "The reviewers observations and interpretations of these studies were in good agreement with the previously reported observations and interpretations..." In answer to issue (d) he states: "There was no evidence of a cancerous or precancerous response to in either species." addressed issue (c) with his discussion of the findings in rats and dogs as quoted below and provides the following overall conclusion:

> "It was concluded that (a) all treatment related changes observed were the consequence of local irritation, with associated protective responses normally seen with irritant materials in the respiratory tract of laboratory animals, (b) there were distinct dose-response relationships with clear interspecies differences, and (c) interspecies differences in local dosimetry should be considered when addressing humans health risk concerns for the formulations used in these studies."

With respect to the findings in rats, states:

> "Significant vehicle effects were observed in the larynx of rats, especially with respect to necrosis of the U-shaped cartilage, and these responses were exacerbated by exposure to The larynges of rats exposed to the vehicle alone (males) and ( (males and females) also exhibited epithelial responses to treatment, including epithelial hyperplasia (with or without minimal, focal keratinization) and squamous metaplasia (without keratinization). These epithelial changes occurred with a clear dose response-relationship, with moderate changes at the high dose, minimal responses at the intermediate dose, and little or no-evidence of toxicity at the low dose. A recovery study demonstrated rapid repair of laryngeal epithelial changes, while focal degeneration of the laryngeal U-shaped cartilage, which was present in all groups exposed to the vehicle, with or without did not recover. The sensitivity of the U-shaped laryngeal cartilage in the rat, a structure which does not have a homologue in man, was attributable to species specific anatomy. This laryngeal response in rats is considered to be of doubtful significance with respect to concerns for human health during exposure to . Rats were

clearly more sensitive to formulations than were dogs."

With respect to findings in dogs, states:

"Potentially treatment-related changes in dogs were confined to two females at the high dose of in study D13342. The responses in these two dogs were very different from each other in nature, and, though it was not possible to exclude a treatment relationship, they were considered to be of little or no toxicological significance."

The details o discussion will not be reproduced here, since a copy of his full review is appended to this document. Some procedural sections are noted below for further discussion. It should be noted that throughout his consultation, [ refers to all of the studies as inhalation studies, when only one of the four studies he looked at was an inhalation study (# R12282 in rats). The other three studies were all intranasal studies (# D13342, #D13901, #D12787 in dogs).

Portions of four of the preclinical studies were assessed by as delineated below. These studies include the studies considered pivotal by the Agency. However, it should be noted that the following studies were not assessed by the consultant: (1) study # R12285 - a pilot 8 day inhalation toxicity study in AHA rats, (2) study # P11224 - a 14 day intranasal irritancy study in cynomolgus monkeys and (3) study # D12279 - a one month intranasal toxicity study in beagle dogs (using the preserved formulation), as well as several minor studies which were not considered to be of primary importance to the Agency (study # D12278 - Pilot study to determine the maximum repeatable daily intranasal dosage level in dogs-preserved formulation, studies # 110973, 110974 and 110975 three different one week intranasal irritancy studies in dogs using a gel based formulation).

1. Study R12282 - the 35 day inhalation study in rats (using the preserved formulation).
   From the report it appears that the examination of the rat study was the most complete of the consultation. For this study, the consultant states: "....the following procedure was adopted: for all male rats in the main study I examined all slides from the respiratory tract, including nasal passages, buccal cavity/oropharynx, larynx (multiple step sections), trachea and bronchial bifurcation, lungs, and mediastinal lymph nodes. I then screened the female rats, to confirm the reported similarity between the sexes, and proceeded to review in depth significant target tissues in all female rats; in this case the larynges were examined in detail, as were all other potentially significant endpoints indicated in the accompanying report (minor nasal lesions, deciliation at bronchial bifurcation)."

2. Study D13342 - the 13 week study in dogs using the sweetened (saccharin) formulation.
   In the report states that he re-examined (1) the entire respiratory tract from the first two control males and females, (2) nasal lesions in two high dose treated females exhibiting possibly treatment related lesions, (3) nasal

passages from all high dose treated dogs, (4) all lungs from dogs noted by original pathologist with fibrosing alveolitis.

3. Study D13901 - the 14 day study in dogs using the clinical formulation (buffered).
In the report states that he re-examined entire respiratory tract from all high dose treated dogs only.

4. Study D12787 - the 13 week study in dogs using the unbuffered formulation.
In the report states that he re-examined entire respiratory tract from all high dose treated dogs only.

Summary and Evaluation:

The main purpose of this review is to determine whether the sponsor has adequately addressed the preclinical issues delineated in the 8/28/96 not approvable letter issued by the Agency. The reviewer's objections to the approval of a chronic intermittent use drug, without the appropriate chronic toxicity battery was discussed thoroughly in the Pharmacologist's Original Summary and Review of NDA 20-626, dated 4/15/96.

In their response to the Agency's not approvable letter, the sponsor has supplied further histopathology information about the results of their previously submitted preclinical studies. This further interpretation was based, at least in part, on an independent pathology consultation by B.V.Sc., Ph.D. Diplomate A.C.V.P., F.R.C. Path., whose report, in its entirety is appended to this review (appendix 1).

With reference to the pathology consultation review conducted on behalf of the sponsor by it is not clear to the reviewer; whether the procedure followed by the consultant provides an objective and independent interpretation of the data; however, it does provide a valuable academic interpretation of the information. It should be noted that states: "My objective was not to re-read the four studies but to assess the quality of the original study pathologist(s) interpretations and to draw my own conclusions on treatment-related changes." It should be emphasized that this consultation does not reflect a 'blinded' reading of the slides, and, in some cases, no control animals were examined. It should also be noted that throughout his review refers to all of the studies as inhalation studies, when in fact only one of the four studies he looked at was an inhalation study (# R12282 in rats). The other three studies were all intranasal studies (# D13342, #D13901, #D12787 in dogs). How this possible misunderstanding may have affected the interpretation of any finding cannot be assessed. Therefore, the value of this consultation, for the interpretation of the potentially treatment related findings, is not clear to the reviewer.

Our original plan was for Dr. McKinney, the Agency's consulting Veterinary Pathologist, to examine the slides from several predesignated studies in a 'blinded' fashion, to get a completely independent interpretation of the data. Unfortunately, due to the Agency's severe time constraints, this was not possible. Dr. McKinney initially reviewed the original study protocols and original pathologists' reports, as well as the full report from , the sponsor's consultant. Based on the information available, it was determined that detailed explanation of the results of the 35 day inhalation study in rats was sufficient to answer the kinds of questions we had about the study; however, in order to address our questions about the respiratory findings in the intranasal studies in dogs, Dr. McKinney would need the opportunity to examine tissue slides from studies # D13901, a two week intranasal study in dogs with the clinical formulation, # D12787, a 13 week intranasal study in dogs with the unbuffered formulation, and # D13342, a 13 week intranasal study in dogs with the sweetened formulation. Dr. McKinney examined the slides on 2/17/97. On 2/18/97 the reviewing Pharmacologist and Dr. Fitzgerald, the Pharmacology Team Leader, discussed with Dr. McKinney, at some length, her explanation of the findings from these studies and the

limitations of further interpretation.

At the time that this review was finalized, there was no written documentation of this consultation with Dr. McKinney; however, the reviewer faxed a draft (2/20/97) to Dr. McKinney, for concurrence and accuracy of the opinions expressed. Her editorial corrections were returned by fax on 2/21/97 and her corrections incorporated into the review on 2/24/97. A written summary of the consultation will be supplied by Dr. McKinney and submitted to the file.

Evaluation of the Response to the First Issue of the Not Approvable Letter

The first issue of the not approvable letter (see pages 1-2 of this review for verbatim listings) in essence deals with the significance of the findings of metaplasia in the preclinical studies, and the fact that further interpretation is impossible without a more detailed report of the pathology. Recall that metaplasia occurred in the nasal and laryngeal epithelium of rats after 3, 8 or 35 days of inhalation treatment with a preserved formulation and in the bronchial epithelium of one dog after 14 days on intranasal treatment with clinical formulation. Please see appendix 2 for reviewer supplied summary tables of potentially treatment related pathology and appendix 3 for sponsor supplied tables of lung pathology from the intranasal studies in dogs. The sponsor has provided further details of the pathology as queried, and discussed above.

With reference to the inhalation studies in rats:

a. Squamous metaplasia of the lining of the ventral meatus was demonstrated in one rat (of 10) treated with an estimated daily exposure level of 2.6 mg/cm$^2$ (compared to estimated maximum daily human exposure of 0.5 mg/cm$^2$). According to the sponsor the daily duration of dosing was 125 minutes. This finding was noted in an animal sacrificed for humane reasons after three days of treatment. This finding was not evident in the remaining nine animals exposed to this dosing regimen for a total of eight days, or, in any rat exposed in the 35 day study up to the maximum daily exposure tested which was 0.69 - 1.2 mg/cm$^2$. According to the sponsor: "Given that this was a single occurrence at a very high dose in the rat, we did not consider this finding of significance in assessing the safety of sumatriptan." No further details of this singular finding were provided. Neither the sponsor's consultant, not the Agency's consultant, Dr. McKinney, reviewed this study.

b. The sponsor contends that since there is no analogous structure to the rat's laryngeal ventral cartilage in humans, the treatment related necrosis was "unlikely to be of any toxicological relevance to man. It was not clear to the reviewer, or to Dr. McKinney, the Agency's consultant, that this was an appropriate conclusion to draw. Dr. McKinney explained that due to the airflow pattern in the rat and the anatomical position of this tissue, it could serve as a very sensitive warning system for treatment related toxicity. What this study did demonstrate is that inhalation treatment with the vehicle resulted in necrosis of

the laryngeal cartilage of rats and that the presence of slightly increased the incidence and increased the severity of this finding. It is unfortunate that the sponsor did not carry out an inhalation study in rats using a non-preserved formulation, so that the toxicity of the drug could be separated from the obviously toxic vehicle.

c. The sponsor states that the treatment related changes in the epithelium of the rat larynx consisted of: (1) hyperplasia of the ventrolateral epithelium (normally cuboidal) with occasional squamous metaplasia, (2) hyperplasia of the ventral epithelium (normally squamous) with occasional keratinization, (3) hyperplasia of lateral epithelium (normally squamous), (4) hyperplasia of medial (inner) surfaces of arytenoid processes (normally squamous) with occasional keratinization. The sponsor further summarized that: "Keratinization was confined to the hyperplastic squamous epithelium and squamous metaplasia confined to the cuboidal epithelium; the respiratory epithelium of the larynx (pseudostratified ciliated columnar epithelium) was not affected. Squamous metaplasia with prominent keratinization was not observed in any inhalation or intranasal study with sumatriptan." The sponsor contends that these histopathologic changes are adaptive responses to the inhalation formulation. They further state: "The histological appearance of these findings was of a benign and orderly nature; there was no evidence of any cellular atypia or disorganization which could indicate pre-neoplasia." The sponsor suggests that the degree of recovery demonstrated after 14 days with no treatment further supports the contention: "...that the response observed in both the squamous and cuboidal epithelia of the larynx represents an adaptive defense mechanism where a susceptible epithelium is replaced by a more resistant type." The sponsor also states that the lack of a treatment related histopathology in the 14 day intranasal study in cynomolgus monkeys and a 91 day intranasal study in dogs "suggests that the rat larynx over-predicts as a model for human respiratory tract irritancy, and that the epithelial changes in the rat larynx are of no clinical significance."

Dr. McKinney reviewed the sponsor's original histopathology report, as well as consultation, and essentially agreed that the kinds of findings present at necropsy, which were described in detail by did not portend pre-neoplastic changes. Dr. McKinney explained that the type of squamous metaplasia which would warrant our concern (i.e., nonsquamous cells replaced by squamous cells, which then keratinize) was not present. She agreed with interpretation that the cellular changes present at necropsy represent an adaptive response to irritation. She stated that we cannot make a statement that further treatment, more chronic treatment, could not produce a different lesion, which may change over time into something more worrisome.

The validity of the sponsor's contention that lack of treatment related effect in the studies in monkeys and dogs "suggests that the rat larynx over-predicts as a model for human respiratory tract irritancy, and that the epithelial changes in the

rat larynx are of no clinical significance" is unclear. The studies should be considered as separate entities. First, there is the issue of comparing the routes of administration. The studies conducted in rat were carried out by the inhalation route. The Agency's Division of Pulmonary Drug Products has a history of accepting studies by the nasal inhalation route in rats to support intranasal drug use, since rats are obligatory nose breathers, and thus, the nasal cavity would be exposed. All of the studies conducted in dogs and monkeys used the intranasal route of administration. The inhalation studies were designed to deliver an aerosol concentration of [redacted] base of approximately 1.3 mg/l, (i.e., 0.001mg/ml) over variable exposure periods (in the main study 15 - 60 minutes daily). As part of the intranasal studies in dogs, animals were exposed to drug concentrations of 200 to 400 mg [redacted] base/ml, as short duration sprays, two (in dogs) to four (in monkeys) times daily. It is possible that irritation and local toxicity would be more dependent on multiple short duration exposures to higher concentrations, and thus the rat inhalation model might under predict certain local toxicity. The proposed regimen for humans employs a maximum concentration of 200 mg/ [redacted] base/ml, administered as short-duration sprays twice daily maximum. Second, there is the issue that different vehicles were used in the different studies. The inhalation study in rats employed a preserved vehicle, which produced vehicle-related toxicity in the larynx. The sponsor did not carryout an additional study with a more appropriate vehicle. The intranasal studies in dogs employed a variety of formulations (vehicles). The sponsor did not request that their consultant review study #D12279, the one month intranasal study in dogs with the preserved formulation; however, this study was not considered pivotal by the Agency. Third, there is some disagreement with both the sponsor's and [redacted] interpretation of the dogs studies. While there did not appear to be any notable treatment related toxicities in the larynx of the dog or monkey (as were seen in the rat), there were possible treatment related histopathologies of the lung in the three major intranasal studies in dogs (D13901 - 2 week study with the clinical formulation, D12787 - 13 week study with the unbuffered formulation, D13342 - 13 week study with the sweetened formulation) as discussed in detail in the next section. Finally, it seems that the appropriate conclusion is that the studies in rats and dogs show treatment related toxicity to the respiratory system, when administered by the intranasal route (dogs) or the inhalation route (rats).

With reference to the intranasal studies in dogs:

a. In study # D13901, a two week intranasal study in dogs, the only study conducted with the clinical formulation, there was a single incidence in the treated group of focal squamous metaplasia of the bronchial epithelium. According to the sponsor, this should not be considered a significant treatment related finding because it occurred in only one dog in one study, and, a control animal from a Glaxo Wellcome study carried out at [redacted] has also demonstrated this finding. In the current submission, the sponsor supplied historical control data from 13 week studies in beagles from [redacted] between 1988 and 1996. Of the 88 animal data base

used to generate the historical control incidence, none were noted with focal squamous metaplasia of the bronchial epithelium.

discussed this lesion in detail on page 12 of his review and concluded: "...in this reviewers opinion the lesion was insufficiently altered for a diagnosis of squamous metaplasia. The preferred diagnosis is minimal, focal epithelial hyperplasia, as a stage of epithelial repair from a minor local insult. ....On the basis of the lack of lesions in other dogs, and the lack of similar lesions in respiratory epithelium more proximally in the respiratory tract (which would be expected to receive a higher dose of inhale ), it was determined that this lesion was not related to treatment."

According to Dr. McKinney this lesion suggested a reparative change through a broad zone in several bronchial cross sections, a healing lesion, not of infectious origin. It is her opinion that this type of lesion, as it existed at necropsy, would not become worrisome over time. She stated that we cannot make a statement that further treatment, more chronic treatment, could not produce a different lesion, which may change over time into something more worrisome.

b. According to the sponsor, in study # D13342, a 13 week intranasal study in dogs with the sweetened, unbuffered formulation, the focal epithelial hyperplasia of the maxilloturbinate in one dog administered 480 mg/dog/day should not be considered a significant treatment related finding because it occurred in only one dog in one study, and, has occurred in the vehicle control animals of other studies conducted by Glaxo Wellcome. According to the sponsor, the epithelial erosion in the nasal cavity in another dog in this dose group should not be considered a significant treatment related finding because it occurred in only one dog in one study, and, has occurred in the vehicle control animals of other studies conducted by Glaxo Wellcome.

On page 11 of his review, refers to these findings, in two dogs from a single study, as the only potentially treatment related findings in dogs. The incidence of erosion of the nasal vestibule, he notes as possibly procedural in origin, "a minor traumatic injury, possibly during dosing." The incidence of focal epithelial hyperplasia of the maxilloturbinate, he states could either be background or treatment related, an "early adaptive response to a mild irritant" and he suggests the use of historical background information to help in the determination. Note that no historical control data for this finding were included in the current submission. On page 15 of his report further states:

> "If the epithelial hyperplasia seen in one female high dose dog in study D13342 (Animal number 2881) was a consequence of exposure to its nature deserves further consideration. The study in rats indicates that dose-response relationships and interspecies differences in susceptibility play an important role in respiratory tract responses to the formulations used. The nasal epithelial hyperplasia seen in one dog was morphologically similar to mild chlorine-induced lesions

observed in specific regions of the nasal passages of rats (Wolf et al., 1995) and Rhesus monkeys (Klonne et al. 1987). However, it was demonstrated in a chronic rodent bioassay that this chlorine-induced epithelial hyperplasia does not progress to cancer in the rat (Wolf et al., 1995). It was recently concluded that nasal epithelial hyperplasia in rats and Rhesus monkeys exposed to inhaled chlorine is a consequence of site-specific tissue 'irritation' resulting from local airflow-driven dose patterns (Ibanes et al 1996). Similar mechanisms could account for respiratory tract lesions induced by inhaled in rats. Furthermore, the two lesions observed in the treated dogs in the present studies occurred at the front of the nose, in regions which might be expected to receive the highest dose of droplets from the inhaler. The minimal nature of these lesions in the anterior nose of the dog, and the absence of the lesions more distally in the airways of these animals, is consistent with a role for local dose in respiratory tract responses to these inhaled formulations of However, the very different nature of the two lesions found in these dogs, and their absence in the males in this study and in both sexes in the other two dog studies, leads this reviewer to conclude that these changes in dogs are probably of little consequence for human health risk assessment."

According to Dr. McKinney, the single incidence of epithelial hyperplasia is real and appears to be a single small focus located in an area where the turbinate projects into the nasal cavity, not one of the hidden recesses. It did not occur in any of the controls. She states that the lesion at necropsy appears to be reparative or regenerative, and it is not possible to predict what could happen with more chronic treatment.

observation that the hyperplasia seen in the maxilloturbinbate of the one treated dog looks like the pathology that has been demonstrated with chlorine inhalation studies in both rat and Rhesus monkeys, is very interesting. However, the appropriateness of his inference, that this lesion in the dog probably will not progress to neoplasm with chronic treatment, since chronic inhalation treatment of the rats and monkeys with chlorine did not result in the progression of this lesion to neoplasm, is less clear, since we are assessing the toxicity of a different compound in a different species.

c. According to the sponsor, the fibrosing alveolitis demonstrated in study # D13342, the 13 week intranasal study in dogs with the sweetened formulation should not be considered a significant treatment related finding because it occurred only in females, a treatment related increase was not observed in other studies, and, "All of the remaining features of the lung pathology were of comparable incidence and severity in treated and control groups and, thus, did not exhibit any treatment related effects..."

With reference to the fibrosing alveolitis (which he notes in female dogs in study D13342), states that it is of unlikely toxicological relevance;

however, historical control data should help with further interpretation. In the current submission, the sponsor supplied historical control data from 13 week studies in beagles from between 1988 and 1996. Of the 88 animal data base used to generate the historical control incidence, two were noted with fibrosing alveolitis.

The sponsor's line of reasoning is not clear. The sponsor dismisses the finding of an apparent treatment related increase in fibrosing aveolitis based on three arguments. The first is that in study D13342 it occurred only in females. From an Agency standpoint this is not a reason to dismiss the finding, and, additionally, the study has small group sizes (4/sex/group) and, therefore, the fact that it occurred in only one sex, may be a reflection of the lack of power of the assay by design. Second, the sponsor states that a treatment related increase in fibrosing alveolitis did not occur in other studies. It should be noted that there was also an apparent treatment related increase in incidence of fibrosing alveolitis in study D12787, the 13 week intranasal study in dogs with the unbuffered formulation (based on the original study data: 1/8-control, 1/8-low dose and 3/8-high dose). Note that in this study, the original study pathologist's notations of fibrosing alveolitis were not confined to females. It should be noted that for this study, stated "No changes were observed in the respiratory tract of dogs in study D12787 that were considered to be related to treatment with " Thirdly, the sponsor states that all other lung pathologies were comparable in both incidence and severity in treated and control groups. Again, there is a disagreement between the reviewer and the sponsor about apparent treatment related effects. In both of the 13 week intranasal studies in dogs (D12787 and D13342), there were, by the reviewer's interpretation, apparent treatment related increases in other lung pathologies, i.e., granuloma, pleural fibrosis/adhesions, bronchiolitis associated with low grade epithelial hyperplasia and bronchitis. See appendix 4 for reviewer generated summary tables of the lung pathology noted in the 60-90 week oral toxicity study for sumatriptan in dogs and the 26 week subcutaneous toxicity study for sumatriptan in dogs. Note that there were no high background incidences of fibrosing alveolitis, chronic pneumonia or granuloma.

Dr. McKinney examined the slides of the lungs from animals from the two 13 week intranasal studies in dogs (# D12787 and # D13342), unblinded, and with knowledge of the original pathologists' reports and Dr. Morgan's consultation. For studies # D12787 and D13342 she stated that, in general, the apparent treatment related lung pathology represented an active on-going inflammatory process, with a modest degree of difference in severity between the control and treated groups. She did note that the degree of severity tended to be worse in the high dose group. Dr. McKinney stated that it does not represent a pre-neoplastic process; however, it does suggest that something is happening with treatment. There is evidence of chronic active inflammation in the high dose group. Food and/or other foreign particles are present in the alveoli of the high dose treated animals and not in the control group. Based on the study findings, there is no way to negate this apparent treatment related irritation effect. Dr.

McKinney suggested that it is possible that treatment altered the respiratory physiology in some way, so that the animals do not cough, breath or clear their lungs quite normally. The evidence for this is first, that foreign material got into the alveoli of the high dose treated animals and second, that this material was not cleared out of the lungs. For both studies Dr. McKinney stated that she would use the term 'chronic pneumonia' rather than the term 'fibrosing alveolitis'. She said that this strictly a personal preference; however, the two refer to the same finding(s).

The apparent difference in incidence and, to a lesser degree, severity between control and treated groups may be the result of an under-powered assay, i.e., too few animals treated per group to make more definitive statements, and/or the sacrifice of all of the animals at one time point, rather than at several time points, to see temporal effects.

Dr. McKinney reiterated that although the findings in the lung show no evidence of preneoplasia, if the fibrosing alveolitis were truly treatment related, prolonged treatment could result in increased pulmonary fibrosis. Dr. McKinney is not comfortable eliminating the possibility of treatment related chronic pulmonary pathology, based on the results of the two 13 week intranasal studies in dogs.

d. The sponsor did not address the incidence of lymphoid hyperplasia of larynx in one dog and lymphoid hyperplasia of respiratory region of nasal passage in another dog in the 480 mg/dog/day group from study D12787, the 13 week intranasal study in dogs employing the unbuffered formulation. According to Dr. McKinney, these findings of lymphoid hyperplasia should not necessarily be interpreted as treatment- related lesions.

Evaluation of the Response to the Second Issue of the Not Approvable Letter

The second issue of the not approvable letter deals with the pathology reported in the 35-day rat inhalation study. While it appears to be reversible after a 2-week period, there is no assurance that this would be the case after chronic (at least 6 months) treatment. There is also no assurance that the pathology would not have become more serious or widespread with longer exposure.

The data from the original submission did show a notable degree of reversibility of the treatment related histopathology after a two week recovery period. The sponsor has not (and cannot) provide "assurance that this would be the case after chronic (at least 6 months) treatment" as stated in our comments. In fact, on page 14 of his review, comments on the "potential cancerous nature of rat laryngeal responses." He states:

> "In brief, I saw no evidence of a carcinogenic response in these animals. The latter statement, while based on experience with respiratory tract cancer in many stages of development, is not intended to negate any concerns of the sponsor. The presence of keratinization of airway epithelium is a justifiable concern, as it is

clearly a feature of early squamous cell carcinomas, such as those induced by formaldehyde, a well studied rat nasal carcinogen.... However, the principle hallmarks of cancer were clearly absent in this study, as stated in the results (see above), and the minimal amount of keratin present was not associated with squamous metaplasia, but squamous hyperplasia of an existing squamous epithelial lining. The short duration of study R12282 precludes a definitive statement on the potential carcinogenicity of , as this study is designed to assess irritancy."

With reference to this issue, Dr. McKinney stated that the apparent treatment related lesions, as described in the rat, or as viewed in the dog, show no evidence of pre-cancerous change (i.e., there is no evidence of a progression to a further morphologic change) and, that these lesions, at this point in time, are not worrisome. However, she reiterated that this statement refers to the lesions, as they existed at necropsy, and no definitive statements could be made for any changes in pathology which may occur with further treatment.

Evaluation of the Response to the Third Issue of the Not Approvable Letter

The third issue of the not approvable letter deals with the comparison of the estimated nasal cavity exposure among species. Recall that there is essentially no safety margin for the observed pathology in the preclinical studies submitted, when the maximum daily human exposure is compared to the maximum daily exposure in animals. In this case exposure is defined as dose per surface area of the nasal cavity. The sponsor has provided comparative exposures different from those generated by the reviewer (see the reviewer-generated summary table which follows). There are small differences in the estimates of the nasal surface area, based on literature values, used by the sponsor and the Agency. However, the more notable difference is that it appears that the sponsor has made their comparisons based on a maximum single dose of 20 mg, rather than the maximum daily dose proposed for marketing, of 40 mg (a single dose of 20 mg into one nostril, which can be repeated, if necessary).

There does not seem to be a policy about which comparison should be made: (1) maximum proposed total daily exposure of nasal cavity in humans to total daily exposure of nasal cavity in the animals, or, (2) maximum single exposure of nasal cavity in humans to total daily exposure in animals. Because of the design of the preclinical studies conducted, with multiple daily doses (intranasal) or variable duration of exposure (inhalation), it is not possible to look at the effect of repeated single daily doses. Therefore, it seemed logical to compare 'similar situations' among species, i.e., the total daily exposure in animals compared to the maximum proposed total daily exposure in humans. The most conservative estimate would be based on the situation in which the human administered the maximum daily dose (20 mg delivered into a single nostril, repeated, if necessary, into the same nostril).

The sponsor notes that plasma levels of achieved in dogs administered 480 mg/day for 13 weeks (based on study D12787) were approximately 3,300 times greater than those achieved in humans after a single 20 mg intranasal dose. It is not clear to the reviewer how the sponsor determined this. The mean total $C_{max}$ (from both daily doses, day 35) for dogs administered 480 mg/day in study D12787 (unbuffered formulation) was 4060 ± 1550 ng/ml. The mean $C_{max}$ in humans after a single 20 mg intranasal dose is 16.1 ng/ml. Therefore, in this study animals achieved a $C_{max}$ which is 252 times the $C_{max}$ achieved in humans.

| Comparative Exposures | | | | | | |
|---|---|---|---|---|---|---|
| species | Agency | | | Sponsor | | |
| | exposure | | | exposure | | |
| | total daily dose administered (mg/kg/day) | total daily dose per nasal cavity surface area (mg/cm²) | multiple of projected maximum daily human exposure (based on dose per nasal surface area) | total daily dose administered (mg/kg/day) | total daily dose per nasal cavity surface area (mg/cm²) | multiple of projected maximum daily human exposure (based on dose per nasal surface area) |
| dog | 120 | 1.1 (*) | 2.2 | 120 | 1.08 | 5 |
| | 240 | 1.1 (*) | 2.2 | 240 | 1.08 | 5 |
| | 480 | 2.2 | 4.4 | 480 | 2.17 | 9.9 |
| rat | - | 0.17 - 0.31 | 0.3 - 0.6 | 11.5 | 0.28 | 1.3 |
| | - | 0.35 - 0.64 | 0.7 - 1.3 | 23.3 | 0.56 | 2.5 |
| | - | 0.69 - 1.2 | 1.4 - 2.4 | 45.5 | 1.1 | 5 |
| monkey | 160 | 2.6 (**) | 5.2 (**) | 160 | 6.4 | 14.5 |
| | 320 | 5.2 (**) | 10.4 (**) | 320 | 3.2 | 29 |
| human | 40 | 0.5 | - | 20 | 0.22 | - |

(*) dose of 120 mg/day was administered into one nostril only, while the dose of 240 mg/day was divided into two nostrils

In general the Agency's exposure estimate was based on literature values for surface area of nasal cavity: Gizurarson, S., Animal models for intranasal drug delivery studies: a review article, Acta Pharm. Nord. 2(2):105-122, 1990.

(**) The estimate for the nasal cavity surface area of monkeys is based on a 7 kg monkey of unknown strain. The current irritancy study employed much smaller (3.0 kg) cynomolgus monkeys.

Evaluation of the Response to the Concluding Paragraph for the Pre-Clinical Portion of the Not Approvable Letter

Finally, the last issue from the not approvable letter is the necessity for the sponsor to demonstrate "that there is no toxicity suggestive of proliferative or pre-neoplastic changes observed as a consequence of intranasal Imitrex administration" in the preclinical studies. As stated in the letter, if this can be established, the previously conducted carcinogenicity studies by the oral route may support the intranasal dosage form. If this cannot be established, the sponsor may have to carry out a 6-month intranasal toxicity study with a reversibility phase in the most appropriate species.

The reviewer assumes that this stipulation was made in the spirit of the draft of the most current guidance from the Division of Pulmonary Drugs, which states:

> "While not optimal, carcinogenicity studies by the oral route may be sufficient to support inhalation or intranasal clinical routes when no toxicity suggestive of proliferative or preneoplastic changes, such as metaplasia or hyperplasia, is observed in chronic inhalation or intranasal toxicity studies and when adequate local exposure by the oral route is demonstrated."

The chronic toxicity of sumatriptan has been assessed by the oral route (78 week oral toxicity study in rats and 60-90 week oral toxicity study in dogs, a 78 week oral carcinogenicity study in mice and a 104 week oral carcinogenicity study in rats) and by the subcutaneous route (28 week subcutaneous toxicity study in rats and six month subcutaneous toxicity study in dogs). None of these studies by design assessed the local toxicity of intranasal sumatriptan to nasal and respiratory tissues. It should be reiterated that the sponsor has not submitted any chronic intranasal toxicity studies for sumatriptan. According to current Agency standards this would be a six month study in rodents and twelve month study in non-rodents. The most current guidance from the Division of Pulmonary Drugs allows for a certain degree of flexibility for development of a drug by the intranasal or inhalation route, if chronic toxicity has been assessed in two species by another route. Under this condition, the Division of Pulmonary Drugs may accept a single six month intranasal or inhalation study in the most appropriate species, for the assessment of chronic toxicity.

, the pathology consultant for the sponsor, states that based on the tissues he examined: "There was no evidence of a cancerous or precancerous response to in either species." Dr. McKinney, the Agency's pathology consultant, has not noted any preneoplastic or precancerous lesions in any of the tissues which she examined or in any of the preclinical reports she has read for her consultation.

There was evidence of treatment related proliferative (hyperplastic) changes for sumatriptan administered to rats by the inhalation route and dogs by the intranasal route. There were clearly treatment related increases in incidence and severity of laryngeal epithelial hyperplasia and squamous metaplasia in rats after 8 or 35 days of inhalation exposure and in epithelial keratinization in rats after 35 days of inhalation exposure. The scattered, rather isolated incidences of epithelial hyperplasia and squamous metaplasia demonstrated in the intranasal studies in dogs (2 to 13 weeks in duration) are less clearly, though still possibly, related to treatment.

As addressed previously in this section (in the answer to second issue of the Agency's not approvable letter), the sum total of information provided by the preclinical studies does not allow for a definitive statement about the potential for more serious toxicities with chronic administration. To the reviewer there remains notable uncertainty about whether, with increasing duration of treatment, the previously discussed toxicities may progress in degree of severity.

Conclusions:

The sum total of the information in the original package and the supplements does not support approval. This Division has considered drugs for the acute treatment of migraine to be chronic - intermittent use drugs, following the preclinical requirements for chronic use drugs. The sponsor has not, and cannot, adequately address the preclinical issues raised in the not approvable letter without chronic toxicity studies with the clinical formulation.

As stated in the conclusion section (page 70) of the Pharmacologist's Original Summary and Review of NDA 20-626:

> "For sumatriptan, only one intranasal irritancy study with the clinical formulation has been submitted. This was a 14 day intranasal irritancy study in dogs. The sponsor also supplied the report of a small study in rabbits carried out with the clinical formulation to determine the potential for ocular irritancy.
>
> The sponsor has provided several other intranasal irritancy and/or intranasal toxicity studies in dogs using test formulations which were not equivalent to the clinical formulation, which is buffered. These studies employed, unbuffered solutions, unbuffered - sweetened (saccharin) solutions, unbuffered - preserved solutions (not reviewed), and a nasal gel formulation (also not reviewed). The studies employing the unbuffered and unbuffered-sweetened solutions were 13 weeks in duration. The sponsor has provided a very small 14 day intranasal irritancy study in monkeys employing the unbufferd formulation. The sponsor also carried out two inhalation toxicity studies in rats, a pilot 8 day study and 35 day study, using the unbuffered-preserved formulation.
>
> The longest duration study for dogs was 13 weeks, for rats was 35 days and for monkeys was 14 days. For the intranasal studies in dogs and monkeys, animals were treated with the test formulations two to four times per day. For the inhalation studies in rats, animals were exposed (snout only) to the test formulations daily for periods of 15 minutes to two hours. None of these employed the clinical formulation. None of these studies alone, or considered in total, is of sufficient duration to support chronic use in humans. Each one of the studies reviewed, except the two week intranasal study in monkeys, demonstrated some signal of treatment related hyperplasia or metaplasia of the nasal cavity or respiratory organs. This warrants a more thorough preclinical examination of sumatriptan by the intranasal route prior to marketing, especially since histopathology is not available from humans and that this drug is already marketed for migraine by two other routes (oral and subcutaneous)."

It is not clear to the reviewer that the 13 week intranasal study with the unbuffered formulation (D12787) and the 13 week intranasal study with the sweetened-unbuffered formulation (D13342) are appropriate predictors for toxicity of the clinical buffered formulation. Since there are no equivalent studies with the clinical formulation, apparent treatment related pathologies demonstrated with the other formulations

cannot be dismissed. A reanalysis of the data from the original submission demonstrates that there is either something about the clinical formulation, or the slightly different dosing regimen used with the clinical formulation (6 sprays into one nostril per session rather than 3 sprays per nostril per session), which results in higher plasma concentrations one hour after the first dosing session and, a more severe profile of clinical signs. Because of differences in the protocols for the three studies, the plasma drug concentration at one hour post first dose was the only relevant toxicokinetic parameter at comparable doses, which was common to all three studies. It should be emphasized that the sponsor considered the treatment related clinical signs demonstrated with the first dosing session of the clinical formulation to be so severe, that the dosing regimen was halved for the duration of the study, whereas those seen with the two other formulations did not preclude further dosing for 13 weeks, nor preclude dosing for 13 weeks at a dose and concentration twice as high as the clinical formulation.

Note that in the reviewer-generated summary table below, the similarly shaded rows are for those doses and concentrations which are comparable across studies. Note that the final pH of the different formulations was the same (5.4 - 5.6).

| Plasma GR43175 Concentration (ng(base)/ml) | | | | |
|---|---|---|---|---|
| day/time | males | | females | |
| | mean ± S.D. | range | mean ± S.D. | range |
| # D13901 - 14 day study<br>clinical formulation (pH 5.4 - 5.6)<br>dose: 120 mg ; (6 sprays into one nostril)<br>concentration - 200 mg/ml<br>day 1 - 1 hour post dose | 2558 ± 1214 | | 3383 ± 1576 | |
| | Clinical Signs (so severe that dose regimen reduced): extreme pupil dilation, trembling, splayed hindlimbs, unsteady gait, vocalization, subdued behavior | | | |
| # D12787 - 13 week study<br>unbuffered formulation (pH 5.4 - 5.6)<br>dose: 120 mg; (3 sprays/nostril)<br>concentration - 200 mg/ml<br>day 1 - 1 hour post dose | 1484 ± 477 | | 2317 ± 418 | |
| | Clinical Signs: mydriasis, salivation and corneal opacities | | | |
| # D12787 - 13 week study<br>unbuffered formulation (pH 5.4-5.6)<br>dose: 240 mg; (3 sprays/nostril)<br>concentration - 400 mg/ml<br>day 1 - 1 hour post dose | 3533 ± 1228 | | 3768 ± 860 | |
| | Clinical Signs: mydriasis, salivation and corneal opacities | | | |
| # D13342 - 13 week study<br>sweetened-unbuffered formulation (pH 5.4 - 5.6)<br>dose: 120 mg ; (3 sprays/nostril)<br>concentration - 200 mg/ml<br>day 1 - 1 hour post dose | 1038 ± 282 | | 1935 ± 554 | |
| | Clinical Signs: mydriasis and salivation and occasional high pitched vocalization, lachrymation, vomiting and ocular opacities | | | |
| # D13342 - 13 week study<br>sweetened-unbuffered formulation (pH 5.4 - 5.6)<br>dose: 240 mg; (3 sprays/nostril)<br>concentration - 480 mg/ml<br>day 1 - 1 hour post dose | 3533 ± 1228 | | 3768 ± 860 | |
| | Clinical Signs: mydriasis and salivation and occasional high pitched vocalization, lachrymation, vomiting and ocular opacities | | | |
| 14 day study: .<br>13 week studie: | | | | |

Recommendations:

The preclinical toxicology information provided in the original NDA submission and the supplements does not support approval.

1. It is still the recommendation of this reviewer that the sponsor investigate the chronic toxicity of the clinical formulation by the intranasal route, in at least one species (though, ideally two). If it is determined that a single six month study in one species is deemed appropriate, the sponsor should provide a strong rationale for their choice of species. The results of the preclinical chronic toxicity study(ies) by the intranasal route, would determine whether or not carcinogenicity studies by the intranasal route would be required.

2. The outstanding issues from the Pharmacologist's Original Summary and Review of NDA 20-626 (4/15/96) should be addressed. These were issues of review and were not specifically addressed in the not approvable letter.

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Andrea M. Powell

Andrea M. Powell, Ph.D.

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# APPENDIX 1

17 pages
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Information

# APPENDIX 2

Table 1: Histopathology Summary from Intranasal Studies

| species | study # | formulation | concentration mg(base)/ml | dosing regimen | maximum daily total dose mg(base) | maximum total daily exposure estimate (dose/nasal surface area) ($mg/cm^2$) | effects |
|---|---|---|---|---|---|---|---|
| dog 14 day | D13901 | buffered (clinical) | 200 | 0.3 ml, b.i.d. ( 1 nostril dosed) | 120 | 1.1 | 1/8 focal squamous metaplasia of bronchial epithelium (grade 1) |
| dog 13 week | D12787 | unbuffered | 0 | 0.6 ml, b.i.d. (2 nostrils dosed) | 0 | - | 1/8 fibrosing alveolitis (slight focal) |
| | | | 200 | | 240 | 1.1 | 1/8 bronchial lymph node - hyperplasia<br>1/8 fibrosing alveolitis (minimal)<br>2/8 bronchitis (slight - minimal) |
| | | | 400 | | 480 | 2.2 | 3/8 fibrosing alveolitis (slight - minimal)<br>2/8 bronchitis (slight - moderately severe)<br>2/8 granuloma<br>1/8 lymphoid hyperplasia-larynx (minimal)<br>1/8 lymphoid hyperplasia of respiratory region of nasal passages (minimal) |
| dog 13 week | D13342 | sweetened | 0 | 0.6 ml, b.i.d. (2 nostrils dosed) | 0 | - | 1/8 fibrosing alveolitis (minimal)<br>1/8 granuloma (lung)<br>1/8 reactive bronchial lymph node (minimal) |
| | | | 200 | | 240 | 1.1 | 2/8 fibrosing alveolitis (minimal)<br>3/8 granuloma (lung) (*) |
| | | | 400 | | 480 | 2.2 | 4/8 fibrosing alveolitis (minimal)<br>1/8 pleural fibrosis/adhesion (moderate - multifocal)<br>4/8 granuloma (lung) (*)<br>1/8 bronchiolitis associated with low grade epithelial hyperplasia<br>3/8 reactive bronchial lymph node (minimal)<br>1/8 focal erosion of nasal cavity (minimal)<br>1/8 epithelial hyperplasia (maxilloturbinates) (minimal) |
| dog 1 month | D12279 | preserved | 0 (water) | 0.6 ml, b.i.d. (2 nostrils dosed) | 0 | 0 | Histopathology was not formally reviewed. The sponsor did not submit the standard summary tables. Findings are reported in the 30 individual animal reports from necropsy. |
| | | | 0 (vehicle) | | 0 | 0 | |
| | | | 10 | | 12 | 0.05 | |
| | | | 63.3 | | 76 | 0.3 | |
| | | | 400 | | 480 | 2.2 | |
| monkey 14 day | P11224 | unbuffered | 200 | 0.2 ml, q.i.d. (2 nostrils dosed) | 160 | 2.6 | - 1/4 foci of alveolar macrophage |
| | | | 400 | | 320 | 5.2 | 1/4 focal pulmonary fibrosis and pleural adhesion |
| human | proposed | buffered (clinical) | 200 (maxi mum) | 0.1 ml, b.i.d. (maximum) (1 nostril dosed) | 40 | 0.5 | - |

(*) dose related increase in degree affected

Exposure estimate based on literature values for surface area of the nasal cavity: Gizurarson, S., Animal models for intranasal drug delivery studies: a review article, Acta Pharm. Nord. 2(2):105-122, 1990. Note that the estimate for the nasal cavity surface area in monkeys is based on a 7 kg monkey of unknown strain. The toxicity study in monkeys employs the much smaller (< 3.0 kg) cynomolgus monkeys.

Note "a" - This study had a vehicle control group consisting of 4 males + 4 females

Note "b" - This study had a vehicle control group consisting of 2 males + 2 females

BASED ON 3 DAYS OF EXPOSURE: (Study # R 12285)

| Table 2a: Histopathology Results from Preliminary Study in Rats | | |
|---|---|---|
| | | 3 days of treatment |
| hours/day of exposure to test formulation (or vehicle) | | 2 |
| lung | foamy macrophage aggregate | 1/1 |
| lung | alveolar hemorrhage | 1/1 |
| larynx | widespread submucosal acute inflammation | 1/1 |
| larynx | epithelial hyperplasia of ventral region | 1/1 |
| larynx | ulceration in ventral region | 1/1 |
| nasal cavity | squamous metaplasia lining of the ventral meatus | 1/1 |

Maximum total daily dose exposure estimate (dose/nasal cavity surface area ) for this animal is 2.6 mg/cm$^2$
Maximum total daily dose exposure estimate (dose/nasal cavity surface area) for humans is 0.5 mg/cm$^2$

Exposure estimate based on literature values for surface area of the nasal cavity: Gizurarson, S., Animal models for intranasal drug delivery studies: a review article, Acta Pharm. Nord. 2(2):105-122, 1990.

BASED ON 8 DAYS OF EXPOSURE (Study # R 12285)

| Table 2b: Histopathology Results from Preliminary Study in Rats | | | | |
|---|---|---|---|---|
| | | 8 days of treatment | | |
| | | vehicle | low dose | high dose |
| hours/day of exposure to test formulation (or vehicle) | | 2 | 1 | 2 |
| Maximum total daily dose exposure estimate (dose/nasal cavity surface area) (mg/cm$^2$) | | 0 | 0.9 - 1.2 | 1.8 - 2.6 |
| lung | foamy macrophage aggregate | 0/10 | 1/10 | 1/9 |
| larynx | submucosal inflammatory cell infiltrate | 1/10 | 0/10 | 3/8 |
| larynx | epithelial hyperplasia on arytenoid process | 0/10 | 9/10 | 6/8 |
| larynx | epithelial hyperplasia (ventral region) | 0/10 | 1/10 | 7/8 |
| larynx | squamous metaplasia | 0/10 | 2/10 | 6/8 |
| larynx | mononuclear cell focus in ventral pouch | 0/10 | 0/10 | 1/8 |
| larynx | small glandular cyst(s) ventral pouch | 0/10 | 2/10 | 0/8 |

Maximum total daily dose exposure estimate (dose/nasal cavity surface area) for humans is 0.5 mg/cm$^2$

Exposure estimate based on literature values for surface area of the nasal cavity: Gizurarson, S., Animal models for intranasal drug delivery studies: a review article, Acta Pharm. Nord. 2(2):105-122, 1990.

BASED ON 35 DAYS OF EXPOSURE (STUDY # R12282)

Table 3: Histopathology Results from Main Study in Rats

| | 35 days of treatment | | | | | 35 days of treatment + 2 week recovery period | | |
|---|---|---|---|---|---|---|---|---|
| | air control | vehicle control | low dose | mid dose | high dose | air control | vehicle control | high dose |
| hours exposure to test formulation (or vehicle or air) | 1 | 1 | 0.25 | 0.5 | 1 | 1 | 1 | 1 |
| total daily dose exposure estimate (dose/nasal cavity surface area) ($mg/cm^2$) | 0 | 0 | 0.17 - 0.31 | 0.35 - 0.64 | 0.69 - 1.2 | 0 | 0 | 0.69 - 1.2 |
| larynx necrosis of the ventral cartilage | 0/20 | 17/20 | 9/20 | 18/20 | 20/20 | 0/10 | 8/10 | 10/10 |
| larynx ventral epithelium | | | | | | | | |
| - hyperplasia | 0/20 | 7/20 | 10/20 | 15/20 | 17/20 | 0/10 | 2/10 | 0/10 |
| - keratinization | 0/20 | 2/20 | 0/20 | 2/20 | 8/20 | 0/10 | 0/10 | 0/10 |
| larynx ventrolateral epithelium | | | | | | | | |
| - hyperplasia | 0/20 | 0/20 | 2/20 | 5/20 | 0/20 | 1/10 | 1/10 | 3/10 |
| - hyperplasia with squamous metaplasia | 0/20 | 0/20 | 2/20 | 2/20 | 14/20 | 0/10 | 0/10 | 0/10 |
| larynx lateral epithelium | | | | | | | | |
| - hyperplasia | 0/20 | 0/20 | 0/20 | 7/20 | 12/20 | 0/10 | 0/10 | 0/10 |
| - keratinization | 0/20 | 0/20 | 0/20 | 0/20 | 7/20 | 0/10 | 0/10 | 0/10 |
| larynx epithelium of the arytenoid projection | | | | | | | | |
| - hyperplasia | 9/20 | 7/20 | 18/20 | 16/20 | 18/20 | 5/10 | 5/10 | 5/10 |
| - keratinization | 1/20 | 0/20 | 2/20 | 10/20 | 13/20 | 0/10 | 0/10 | 0/10 |
| tracheal bifurcation epithelium | | | | | | | | |
| - apparent loss of cilia | 1/20 | 0/20 | 1/20 | 0/20 | 3/20 | 1/10 | 1/10 | 1/10 |

Maximum total daily dose exposure estimate (dose/nasal cavity surface area) for humans is 0.5 $mg/cm^2$[1]

Exposure estimate based on literature values for surface area of the nasal cavity: Gizurarson, S., Animal models for intranasal drug delivery studies: a review article, Acta Pharm. Nord. 2(2):105-122, 1990.

# APPENDIX 3

# FINDINGS IN THE LUNGS

STUDY NO.: D13901
TREATMENT DURATION: 2 Weeks

| | M | M | F | F |
|---|---|---|---|---|
| Group | 1 | 2 | 1 | 2 |
| Dose (mg/animal/day) | 0 | 120 | 0 | 120 |
| No. Dogs/Group | 4 | 4 | 4 | 4 |
| **PNEUMONIA/ITIS** | 4 | 4 | 4 | 3 |
| **FOCAL ALVEOL MACROPHAGES** | 2 | 1 | 1 | 1 |
| **CONGESTION/HAEMORRHAGE** | 0 | 1 | 0 | 0 |
| **FOCAL SQUAMOUS METAPLASIA** | 0 | 0 | 0 | 1 |

STUDY NO.: D12787
TREATMENT DURATION: 13 Weeks

| | M | M | M | F | F | F |
|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 1 | 2 | 3 |
| Dose (mg/animal/day) | 0 | 240 | 480 | 0 | 240 | 480 |
| No. Dogs/Group | 4 | 4 | 4 | 4 | 4 | 4 |
| **ECTOPIC BONE** | 0 | 0 | 0 | 0 | 0 | 1 |
| **PIGMENTED HISTIOCYTES** | 1 | 0 | 0 | 0 | 0 | 0 |
| **FIBROSING ALVEOLITIS** | 1 | 1 | 2 | 0 | 0 | 1 |
| **GRANULOMA** | 0 | 0 | 1 | 0 | 0 | 1 |
| **LEUKOCYTE FOCI** | 4 | 2 | 4 | 4 | 4 | 3 |
| **BRONCHITIS** | 0 | 2 | 1 | 0 | 0 | 1 |
| **PNEUMONITIS** | 2 | 1 | 1 | 1 | 0 | 0 |

STUDY NO.: D13342
TREATMENT DURATION: 13 Weeks

| | M | M | M | F | F | F |
|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 1 | 2 | 3 |
| Dose (mg/animal/day) | 0 | 240 | 480 | 0 | 240 | 480 |
| No. Dogs/Group | 4 | 4 | 4 | 4 | 4 | 4 |
| **ECTOPIC BONE** | 0 | 0 | 0 | 0 | 1 | 0 |
| **PIGMENTED HISTIOCYTES** | 0 | 1 | 0 | 0 | 0 | 0 |
| **FIBROSING ALVEOLITIS** | 0 | 0 | 0 | 1 | 2 | 4 |
| **FOAMY HISTIOCYTES** | 0 | 1 | 0 | 1 | 0 | 0 |
| **OEDEMA** | 3 | 1 | 1 | 1 | 1 | 2 |
| **HAEMORRHAGE** | 0 | 4 | 1 | 2 | 2 | 2 |
| **MICROTHROMBUS** | 0 | 0 | 0 | 1 | 0 | 0 |
| **VASCULITIS** | 0 | 0 | 0 | 1 | 1 | 0 |
| **PLEURAL FIBROSIS/ADHESION** | 0 | 0 | 1 | 0 | 0 | 0 |
| **GRANULOMA** | 1 | 2 | 2 | 0 | 1 | 2 |
| **INFLAMMATORY CELL FOCI** | 4 | 3 | 3 | 1 | 3 | 2 |
| **BRONCHITIS/BRONCHIOLITIS** | 3 | 3 | 3 | 3 | 4 | 4 |
| **PNEUMONITIS** | 4 | 3 | 4 | 3 | 4 | 4 |

# FINDINGS IN THE UPPER RESPIRATORY TRACT

STUDY NO.: D13901
TREATMENT DURATION: 2 weeks

| | M | M | F | F |
|---|---|---|---|---|
| GROUP | 1 | 2 | 1 | 2 |
| DOSE (mg/animal/day) | 0 | 120 | 0 | 120 |
| NO. DOGS/GROUP | 4 | 4 | 4 | 4 |
| **NASAL CAVITY (POST)** | | | | |
| **ACUTE INFLAMMATORY CELL FOCI** | 0 | 1 | 0 | 0 |
| **NASAL CAVITY (ANT)** | | | | |
| **ACUTE INFLAMMATORY EXUDATE** | 1 | 0 | 1 | 0 |
| **LARYNX** | | | | |
| **LYMPHOID FOCI** | 4 | 3 | 3 | 4 |
| **ACUTE INFLAMMATION** | 0 | 0 | 1 | 0 |
| **TRACHEA** | | | | |
| **INTRA-EP PMN INFILTRATION** | 1 | 1 | 0 | 0 |

STUDY NO.: D12787
TREATMENT DURATION: 13 Weeks

| | M | M | M | F | F | F |
|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 1 | 2 | 3 |
| Dose (mg/animal/day) | 0 | 240 | 480 | 0 | 240 | 480 |
| No. Dogs/Group | 4 | 4 | 4 | 4 | 4 | 4 |
| **NASAL PASSAGES** | | | | | | |
| **HAEMORRHAGE** | 0 | 1 | 0 | 0 | 0 | 0 |
| **LYMPHOID HYPERPLASIA** | 0 | 0 | 0 | 0 | 0 | 1 |
| **LARYNX** | | | | | | |
| **LYMPHOID HYPERPLASIA** | 0 | 0 | 1 | 0 | 0 | 0 |

STUDY NO.: D13342
TREATMENT DURATION: 13 Weeks

| | M | M | M | F | F | F |
|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 1 | 2 | 3 |
| Dose (mg/animal/day) | 0 | 240 | 480 | 0 | 240 | 480 |
| No. Dogs/Group | 4 | 4 | 4 | 4 | 4 | 4 |
| **NASAL PASSAGES** | | | | | | |
| **CYSTIC GLANDS** | 0 | 0 | 0 | 0 | 1 | 0 |
| **FOCAL EROSION** | 0 | 0 | 0 | 0 | 0 | 1 |
| **EPITHELIAL HYPERPLASIA** | 0 | 0 | 0 | 0 | 0 | 1 |
| **PHARYNX** | | | | | | |
| **INFLAMMATORY FOCI** | 1 | 1 | 1 | 1 | 0 | 0 |
| **LARYNX** | | | | | | |
| **INFLAMMATORY CELL FOCI** | 1 | 2 | 0 | 0 | 2 | 1 |
| **TRACHEA** | | | | | | |
| **INFLAMMATORY CELL FOCI** | 3 | 1 | 2 | 1 | 1 | 0 |
| **FOCUS TRANSITIONAL EPITHELIUM** | 3 | 1 | 2 | 3 | 2 | 4 |

# APPENDIX 4

LUNG HISTOPATHOLOGY FROM 60 - 90 WEEK ORAL STUDY
(study was conducted at Huntingdon Research Center starting in 8/87 with dogs from Glaxo's breeding colony)

| | 60 weeks of treatment | | | | 90 weeks of treatment + 4 weeks recovery | |
|---|---|---|---|---|---|---|
| | 0 mg/kg | 2 mg/kg | 10 mg/kg | 50 mg/kg | 0 mg/kg | 50 mg/kg |
| no abnormalities detected | 9/9 | 8/8 | 7/8 | 7/9 | 2/3 | 3/3 |
| acute pneumonia with multiple gram positive diplococci (marked) | | | | 1/9 (*) | | |
| alveolar wall thickening/fibrosis | | | 1/8 | 1/9 | | |
| pleural thickening/fibrosis | | | 1/8 | 1/9 | | |
| lymphocytic infiltration | | | | | 1/3 | |
| | | | | | | |

(*) early humane sacrifice on 35 due to pneumonia

LUNG HISTOPATHOLOGY FROM SIX MONTH SUBCUTANEOUS STUDY
(study was conducted at Glaxo UK starting in 5/89 with dogs from Glaxo's breeding colony)

| | 26 weeks of treatment | | | | 26 weeks of treatment + 35 day recovery | |
|---|---|---|---|---|---|---|
| | 0 mg/kg | 1 mg/kg | 3.5 mg/kg | 12 mg/kg | 0 mg/kg | 12 mg/kg |
| not examined | | | | | 4/4 | 4/4 |
| no abnormalities detected | 7/8 | 6/8 | 8/8 | 7/8 | | |
| chronic inflammation | | 2/8 | | | | |
| perivascular lymphocytes | 1/8 | | | | | |
| fibrosis | | | | 1/8 | | |
| | | | | | | |

## PHARMACOLOGIST'S ORIGINAL SUMMARY AND REVIEW OF NDA 20-626

Reviewer: Andrea M. Powell, Ph.D.

Date of Review: 4/15/96

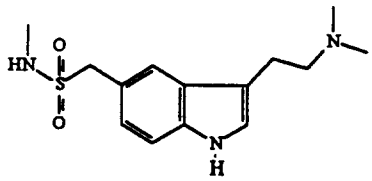
Sponsor: Glaxo Wellcome Inc.
Research Triangle Park, NC 27709

Drug: Sumatriptan, Imitrex® Nasal Spray

Code Number: Nasal Spray

Chemical Name: 3-[2-(dimethylamino)ethyl]-N-methyl-1H-indole-5-methane sulfonamide

Structural Formula:



(the hemisulfate is present *in situ* in the spray formulation)

Molecular Formula: $C_{14}H_{21}N_3O_2S$

Molecular Weight:: 295.4

Pharmacologic Category: agonist of $5HT_1$-like receptor

Indication: acute treatment of migraine headaches

Dosage Form: nasal spray (5 mg, 10 mg and 20 mg)

Clinical Dose: proposed single dose of 20 mg (10 and 5 mg will be available)
maximum daily dose of 40 mg

Related NDAs and INDs:

Note: Portions of this review were excerpted directly from the sponsor's submission.

NDA 20-626

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**Kinetics, Metabolism and Excretion Following Intranasal and Intravenous Administration to Albino Male and Female Rats.**

Testing Facility: (1)

(2) Glaxo Research and Development Ltd., Drug Metabolism II Department

Date of Study: unstated (report issued 5/25/95)

Test Material: (batch # C2596/30/1) and non-radiolabelec (lot # E93K0582, batch # C1893/80/4, Glaxo batch # 32090114) were provided by different departments of Glaxo. Both forms of the drug substance were stored protected from light, with the labeled compound at -80 °C and the non-radiolabeled compound at 4 °C.

The solutions were made up in a potassium/sodium phosphate buffer with the addition of sulphuric acid to form the hemisulfate salt, *in situ* from the base The concentrations of the dosing solutions were 62.5 mg(base)/ml for the intranasal studies and 6.25 mg(base)/ml for the intravenous studies. According to the report, the concentration of the dosing solutions was determined by the sponsor and the mean was found to be within 6% of the nominal value.

Animals: Sprague Dawley rats were chosen, according to the sponsor, to be consistent with previous PK studies. The rats were obtained from and were 7-9 weeks of age at dosing. Animals were housed 3/cage by sex and had free access to food and water, except when removal was necessary for testing. When animals were moved to metabolism cages they were housed singly.

Dosing Regimen: 5 mg(base)/kg, was chosen, according to the sponsor, to be consistent with previous PK studies.

Protocol:

Intranasal Rats were anesthetized (diethyl ether) and dosed with a total dose of 5 mg (base)/kg (approximately 17.7 MBq/kg for radioactive studies). The appropriate volume of the test solution (62.25 mg (base)/ml) was divided into two equal volumes, one per nostril, using a positive displacement pipet. After dosing the head was kept vertical for several moments to prevent loss of dose. Animals were returned to their metabolism cages within 5 minutes of dosing. For non-radiolabeled studies ten groups of 3/sex were used.

Intravenous Rats were anesthetized (diethyl ether), the femoral vein in the right leg exposed through an incision, an injection was made into the femoral vein followed by closure of skin with suture. According to the report all animals were dosed within 12 % of target dose 5 mg(base)/kg (or 17 MBq/kg for radioactive work). Animals were returned to their metabolism cages within 5 minutes of dosing. For non-radiolabeled studies ten groups of 3/sex were used.

Radiolabel studies: Urine and feces were collected from three animals per sex, per route, according to the following schedules:

urine: 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours.

feces: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours.

At the end of the study period animals were sacrificed and the carcasses retained, digested and sampled. The cages were washed and the washes were retained. Debris in the cages were added to the washings.

Plasma was collected from 3/sex/route from an additional set of dosed animals. These animals were maintained under anesthesia and, 10 seconds prior to the first scheduled sampling, the tail tip was cut off. Blood was obtained from the tail. The animals were allowed to regain consciousness. Prior to each additional scheduled sampling period, the scab on the tail was removed and the animal re-bled. Each animal was sampled at 5, 30, 45 minutes, 1, 1.5, 2, 6, 12, 24, 48, 72, and 96 hours post dose. According to the sponsor 96 hours was the termination point because the radioactivity levels dropped below quantitation limits. The blood was stored for up to 6 hours under refrigeration prior to centrifugation. A single control male and female were anesthetized and exsanguinated and used for blanks. PK parameters were determined using the program SIPHAR.

Non-radiolabeled studies: Animals 3/sex/timepoint were sacrificed by exsanguination under anesthesia by cannulation of the dorsal aorta, at 4,15, 30, 45 minutes, 1, 1.5, 2, 6, 12 and 24 hours post treatment. Those sacrificed at 5 minutes post dose were maintained under original anesthesia, all of the others were allowed to recover prior to being reanesthetized and sacrificed. Control animals, 6/sex, were also exsanguinated and sampled. Blood was collected into plastic tubes allowed to clot at room temperature and stored in the refrigerator overnight. Serum was harvested and frozen (-20 °C) and transported to sponsor for analysis. PK parameters were determined using the program SIPHAR.

Results

Plasma Pharmacokinetics of Total Radioactivity and :

Please refer to the following sponsor-supplied summary figures for intravenous and intranasal administration and the sponsor-supplied summary tables of PK parameters. Note that intranasal administration was associated with greater variability than the intravenous administration. The sponsor points out that two of the three males administered radiolabelec intranasally had secondary $C_{max}$ values at 2 hours and at 6 hours post dosing. They speculate that the third male may also have had one, which was missed due to insufficient sampling (by protocol) between 2 and 12 hours. The sponsor also notes this secondary $C_{max}$ between 2 and 6 hours post dose in two of the three females given radiolabelec intranasally. For those animals treated intranasally with non-radiolabeled , the sponsor notes a primary $C_{max}$ at approximately 30 minutes post dose and a secondary $C_{max}$ at approximately 90 minutes post dose. Based on the $AUC_{0\text{-}6\ hours}$ the mean bioavailability of the parent compound by the intranasal route is approximately 30%.

Metabolism: Profiles of urinary radioactivity separated by showed up to seven discrete areas of radioactivity. Urinary metabolism is summarized in the following reviewer generated table. Note that for both intranasal and intravenous administration , the majority of the radioactivity excreted in the urine is as parent compound, followed by the knowr Urine from both routes also have notable levels of the unidentified metabolite (U1). Intranasal administration produces small amounts of

| Major radioactive components in urine (0 - 24 hrs) as % of radioactive dose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| route | sex | total radioactivity as % of dose | parent | | U1 | U2 | U3 | U4 | U5 |
| in | ♂ + ♀ | 76.5 ± 5.66 | 39.2 ± 3.48 | 12.7 ± 4.76 | 10.5 ± 0.91 | 1.18 ± 2.25 | 3.77 ± 3.54 | 2.58 ± 3.73 | 2.69 ± 0.60 |
| iv | ♂ + ♀ | 79.8 ± 6.70 | 49.8 ± 4.76 | 14.0 ± 2.22 | 9.48 ± 1.78 | - | - | - | - |

Some profiles of fecal radioactivity separated by ere provided. For both routes most of the radioactivity was associated with the parent compound, followed by lower amounts of the identified The radioactive profile of the feces after intravenous administration revealed an additional and unidentified metabolite (F1), which was also noted in some intranasal treated animals. According to the sponsor this corresponds to the unidentified urinary metabolite, U1. The example of a typical profile of fecal radioactivity after intravenous dosing also showed another area of radioactivity (U3), which was unidentified.

Excretion: After intranasal and intravenous administration excretion of radioactivity was predominantly urinary, with some fecal component. The following reviewer-generated table summarizes excretion of radioactivity. The sponsor notes that there was somewhat of a difference between routes with reference to time of excretion, with intravenous administration associated with the faster elimination.

| Excretion of Radioactivity (%) over 7 Days | | | | |
|---|---|---|---|---|
| parameter | intravenous | | intranasal | |
| | male | female | male | female |
| urine | 87.0 ± 2.83 | 79.7 ± 5.99 | 85.8 ± 1.06 | 81.9 ± 7.20 |
| feces | 8.69 ± 3.30 | 15.2 ± 3.87 | 18.4 ± 3.18 | 23.1 ± 4.75 |
| cage wash/debris | 0.07 ± 0.01 | 0.10 ± 0.11 | 0.28 ± 0.19 | 0.30 ± 0.02 |
| carcass | 0.60 ± 0.55 | 0.72 ± 0.36 | 0.47 ± 0.05 | 0.52 ± 0.17 |
| total | 96.3 ± 1.45 | 95.7 ± 1.91 | 105 ± 3.00 | 106 ± 2.89 |



APPEARS THIS WAY
ON ORIGINAL

**Table 3** **Summary of plasma radioactivity kinetics following intravenous or intranasal administration of $^{14}$C-GR 43175 to male and female rats at a dose of 5 mg base/kg body weight**

Values are means of n = 3 males and n = 3 females per dose route.

**Intravenous**

| Parameter | Mean males | Mean females | Mean all |
|---|---|---|---|
| Cmax (ng/ml) | 3660 | 3980 | 3783 |
| Tmax (hrs) | 0.083 | 0.083 | 0.083 |
| $AUC_{(0-6\ hrs)}$ (ng.hr/ml) | 5704 | 5505 | 5270 |
| $T_{½}$ (hrs) | 1.7 | 2.0 | 1.85 |

**Intranasal**

| Parameter | Mean males | Mean females | Mean all |
|---|---|---|---|
| $Cmax_1$ (ng/ml) | 396 | 242 | 319 |
| $Tmax_1$ (hrs) | 0.75 | 0.67 | 0.71 |
| $Cmax_2$ (ng/ml) | 314* | 274* | 299 |
| $Tmax_2$ (hrs) | 4* | 4* | 4 |
| $AUC_{(0-6\ hrs)}$(ng.hr/ml) | 1760* | 1290 | 1450 |
| Absorption % 0 - 6 hrs | 30.9 | 23.4 | 27.5 |

For pharmacokinetic parameters of individual rats following intravenous and intranasal dosing refer to Appendix Tables 14 and 15, respectively.

* Average of two animals only

**Table 4** **Summary of pharmacokinetic parameters following intravenous or intranasal administration of GR 43175 to male and female rats at a dose of 5 mg base/kg body weight**

**Intravenous**

| Parameter | Mean males | Mean females | Mean all |
|---|---|---|---|
| Cmax (ng/ml) | 3270 | 3560 | 3420 |
| Tmax (hrs) | 0.083 | 0.083 | 0.083 |
| $AUC_{(0-6\ hrs)}$ (ng.hr/ml) | 2180 | 2210 | 2120 |
| $Cl_s$ (ml/min/kg) | 37.8 | 37.1 | 38.8 |
| $V_d$ (l/kg) | 3.57 | 3.60 | 3.73 |
| $T_{½}$ (hrs) | 1.09 | 1.12 | 1.11 |

**Intranasal**

| Parameter | Mean males | Mean females | Mean all |
|---|---|---|---|
| $Cmax_1$ (ng/ml) | 215 | 175 | 195 |
| $Tmax_1$ (hrs) | 0.5 | 0.5 | 0.5 |
| $Cmax_2$ (ng/ml) | 126 | 175 | 144 |
| $Tmax_2$ (hrs) | 2.0 | 1.5 | 1.5 |
| $AUC_{(0-6\ hrs)}$ (ng.hr/ml) | 654 | 645 | 648 |
| $T_{½}$ (hrs) | 3.95 | 5.94 | 3.98 |
| F (%) | 30.0 | 29.2 | 30.6 |

For pharmacokinetic parameters of individual rats following intravenous and intranasal dosing refer to Appendix Tables 16 and 17, respectively.

1 page

PURGED

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Commercial

Information

**A Study of Absorption, Metabolism and Excretion Following Intravenous and Intranasal Administration to the Dog at 1 mg/kg**

Testing Facil

Dates of Study: 2/9/95 - 7/28/95

Test Material: Radiolabeled: (batch # C 2596/30/1) labeled with [$^{14}$C] at the alpha position of the indole ring was supplied by the sponsor.

Non-radiolabeled: was supplied by the sponsor.

Preparation of formulations: GR43175N (the hemisulfate salt of the base was formed *in situ* in a sodium/potassium phosphate buffer at an approximate pH of 5.5 and concentrations of 10 mg(base)/ml (intravenous) and 60 mg(base)/ml (intranasal) with the addition of sufficient radiolabeled drug to provide 100 $\mu$Ci/ml (intravenous) or 10 $\mu$Ci/kg (intranasal). These formulations were stored at less than 8°C, protected from light.

Animals: Pure bred beagles (3 males and 3 females) from which were 6 - 9 months of age and 5.4 - 7.7 kg at the initiation of the study. Dogs were housed individually and had free access to water and a measured quantity of food.

Dosing Regimen: All animals were given a single intravenous dose of 1 mg(base)/kg at a volume of 0.1 ml/kg, followed by a washout period of approximately 4 weeks. Following the washout period, all dogs were given a single intranasal dose of 1 mg(base)/kg, split equally into each nostril by a positive displacement pipet.

Protocol: Following the intravenous or intranasal doses radioactivity in urine, feces, plasma, cage washings, saliva swabs and cage debris were determined. Blood, urine, feces and saliva were collected according to the following schedule.

Urine: Predose, 0 to 6, 6 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144 and 144 to 168 hours post dose.
Feces: Predose, 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144 and 144 to 168 hours post dose.
Blood: Predose, 5, 15 and 30 minutes, 1, 2, 4, 6, 24, 48, 72, 96, 120, 144 and 168 hours post dose.
Saliva: following intranasal dosing, dogs salivated and the saliva was collected on swabs.

All animals were sacrificed and discarded 168 hours after the intranasal dosing.

Parent compound analysis was carried out on plasma samples collected predose, and at 5, 15, 30 minutes, 1, 2, 4, 6 and 24 hours post dose.

Urinary metabolites were assessed in portions of the urine collected between 0 and 24 hours after dosing.

The commercially available 'TopFit' program was used for non-model dependent PK evaluations.

Results

According to the sponsor: "During the course of the study no toxicological signs were observed in the test animals which could have been attributed to the administration of

Plasma Pharmacokinetics of Total Radioactivity and

Please see the page of sponsor-supplied summary tables which are appended. The following table is an estimate of bioavailability based on the ratio of AUCs after intranasal and intravenous administration of the formulations.

| Bioavailability | | |
|---|---|---|
| | males | females |
| total radioactivity (*) | 106% | 110% |
| | 50% | 50% |
| (*) sponsor estimates a range of 89 - 100% based on individual AUCs rather than the mean | | |

Metabolism: Metabolism was only assessed as a urinary metabolite profile. A plasma metabolite profile was not assessed. Two pages of sponsor-supplied summary information follow. Urinary metabolites were determined in the 0-6 hour and 6-24 hour urine samples. Within 24 hours after dosing 96-98% percent of the urinary excretion of radioactivity is complete. Up to nine peaks (regions) of radioactivity were identified for the intravenous and intranasal studies. The urinary metabolite profiles seem to be qualitatively similar between the two routes. The majority of the radioactivity co-chromatographed with the parent compound and metabolite ( Note that according to the sponsor there was no evidence for the formation of conjugates.

Excretion: The following reviewer-generated table summarizes excretion of radioactivity

| Excretion of Radioactivity (%) | | | | |
|---|---|---|---|---|
| parameter | intravenous | | intranasal | |
| | male | female | male | female |
| urine | 61.63 ± 21.53 | 59.75 ± 26.72 | 64.82 ± 4.761 | 66.09 ± 2.428 |
| feces | 8.481 ± 0.638 | 5.15 ± 1.304 | 15.91 ± 2.186 | 12.88 ± 4.372 |
| cage wash | 18.01 ± 19.12 | 21.177 ± 27.23 | 4.652 ± 1.771 | 4.541 ± 2.558 |
| final cage wash | 0.092 ± 0.089 | 0.044 ± 0.029 | 0.143 ± 0.085 | 0.208 ± 0.128 |
| cage debris | 0.141 ± 0.114 | 0.021 ± 0.036 | 0.037 ± 0.014 | 0.062 ± 0.064 |
| saliva swabs | - | - | 1.076 ± 1.092 | - |
| total | 88.35 ± 1.87 | 86.14 ± 0.52 | 86.65 ± 5.469 | 83.79 ± 4.029 |

## Intravenous

### Radioactivity

**Plasma: Pharmacokinetic parameters of total radioactivity in beagle dogs following a single intravenous administration of at a nominal dose level of 1.0 mg base/kg body weight**

| Animal number | $C_{0\,max}$ (µg equiv/mL) | $T_{\frac{1}{2}}$ (h) | Time range (h) | $AUC_{0-t}$ (µg equiv.h/mL) | $AUC_{\infty}$ (µg equiv.h/mL) |
|---|---|---|---|---|---|
| 1M | 1.448 | 1.917 | 1-6 | 2.021 | 2.272 |
| 2M | 1.205 | 1.649 | 1-6 | 1.599 | 1.744 |
| 3M | 1.259 | 1.639 | 1-6 | 1.878 | 2.037 |
| Mean | 1.304 | 1.735 | NA | 1.833 | 2.018 |
| SD | 0.128 | 0.158 | NA | 0.215 | 0.265 |
| 4F | 1.107 | 2.383 | 1-6 | 2.276 | 2.737 |
| *5F | 1.237 | 1.734 | 1-6 | 1.823 | 1.995 |
| 6F | 1.097 | 2.077 | 1-6 | 2.899 | 2.932 |
| Mean | 1.147 | 2.065 | NA | 2.333 | 2.555 |
| SD | 0.078 | 0.325 | NA | 0.540 | 0.494 |

NA = Not applicable
* 72 h value omitted from PK analysis
t = 6 h (with the exception of 6F, where t = 24 h)

### Parent Compound

**Plasma: Pharmacokinetic parameters of in beagle dogs following a single intravenous administration of at a nominal dose level of 1.0 mg base/kg body weight**

| Animal number | $C_{0\,max}$ (ng/mL) | $T_{\frac{1}{2}}$ (h) | Time range (h) | $AUC_{0-t}$ (ng.h/mL) | $AUC_{\infty}$ (ng.h/mL) |
|---|---|---|---|---|---|
| 1M | 1228 | 1.430 | 0.5-6 | 830.4 | 866.7 |
| 2M | 1010 | 1.261 | 0.5-6 | 653.9 | 672.8 |
| 3M | 1049 | 1.368 | 1-6 | 729.0 | 757.5 |
| Mean | 1096 | 1.353 | NA | 737.8 | 765.7 |
| SD | 116.6 | 0.085 | NA | 88.58 | 97.21 |
| 4F | 915.0 | 1.579 | 0.5-6 | 766.7 | 814.5 |
| 5F | 985.7 | 1.442 | 0.5-6 | 755.5 | 790.8 |
| 6F | 920.3 | 1.525 | 1-6 | 750.8 | 786.0 |
| Mean | 940.3 | 1.515 | NA | 757.7 | 797.1 |
| SD | 39.40 | 0.069 | NA | 8.168 | 15.26 |

NA = Not applicable

## Intranasal

### Radioactivity

**Plasma: Pharmacokinetic parameters of total radioactivity in beagle dogs following a single intranasal administration of at a nominal dose level of 1.0 mg base/kg body weight**

| Animal number | $C_{max}$ (µg equiv/mL) | $T_{max}$ (h) | $T_{\frac{1}{2}}$ (h) | Time range (h) | $AUC_{0-t}$ (µg equiv.h/mL) | $AUC_{\infty}$ (µg equiv.h/mL) |
|---|---|---|---|---|---|---|
| 1M | 0.426 | 1.0 | 4.030 | 2-24 | 1.990 | 2.019 |
| 2M | 0.554 | 1.0 | 4.234 | 2-24 | 2.070 | 2.106 |
| 3M | 0.543 | 1.0 | 4.108 | 2-24 | 2.254 | 2.290 |
| Mean | 0.508 | 1.0 | 4.124 | NA | 2.105 | 2.138 |
| SD | 0.071 | 0.0 | 0.103 | NA | 0.135 | 0.138 |
| 4F | 0.541 | 1.0 | 4.086 | 2-24 | 2.557 | 2.598 |
| 5F | 0.467 | 1.0 | 3.945 | 2-24 | 2.190 | 2.219 |
| 6F | 0.441 | 1.0 | 5.012 | 2-24 | 3.601 | 3.630 |
| Mean | 0.483 | 1.0 | 4.348 | NA | 2.783 | 2.816 |
| SD | 0.052 | 0.0 | 0.580 | NA | 0.732 | 0.730 |

NA = Not applicable
t = 24 h (with the exception of 6F, where t = 96 h)

### Parent Compound

**Plasma: Pharmacokinetic parameters o in beagle dogs following a single intranasal administration at a nominal dose level of 1.0 mg base/kg body weight**

| Animal number | $C_{max}$ (ng /mL) | $T_{max}$ (h) | $T_{\frac{1}{2}}$ (h) | Time range (h) | $AUC_{0-t}$ (ng.h/mL) | $AUC_{\infty}$ (ng.h/mL) |
|---|---|---|---|---|---|---|
| 1M | 128.9 | 0.5 | 2.461 | 1-6 | 268.7 | 327.2 |
| 2M | 134.9 | 0.5 | 2.612 | 2-6 | 342.6 | 422.7 |
| 3M | 197.9 | 0.5 | 2.028 | 2-6 | 345.5 | 388.7 |
| Mean | 153.9 | 0.5 | 2.367 | NA | 318.9 | 379.5 |
| SD | 38.22 | 0.0 | 0.303 | NA | 43.53 | 48.41 |
| 4F | 138.1 | 1.0 | 3.366 | 2-6 | 320.6 | 426.9 |
| 5F | 143.4 | 0.5 | 2.085 | 2-6 | 300.4 | 340.5 |
| 6F | 110.1 | 0.5 | 1.791 | 2-6 | 372.8 | 423.1 |
| Mean | 130.5 | 0.67 | 2.414 | NA | 331.3 | 396.8 |
| SD | 17.89 | 0.29 | 0.837 | NA | 37.36 | 48.82 |

NA = Not applicable

METABOLISM - URINARY - INTRAVENOUS





MALE

Quantitative assessment by [illegible] of radioactivity present in urine following a single intravenous administration o[illegible] to male dogs at a nominal dose level of 1 mg/kg body weight

| Sample | Time point | Region | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1M | 1M | 2M | 2M | 3M | 3M | |
| Urine | 0 to 6 h | U1 | 2.10 | 0.86 | 1.70 | 0.32 | 1.20 | 0.76 | |
| | | U2 | 1.90 | 0.78 | 2.80 | 0.53 | 5.20 | 3.31 | |
| | | U3 | 2.00 | 0.82 | 2.40 | 0.45 | 2.10 | 1.34 | |
| | | U4 | 12.00 | 4.92 | 29.70 | 5.62 | 0.60 | 0.38 | |
| | | U5 | 23.20 | 9.51 | 11.00 | 2.08 | 38.50 | 24.51 | GR49336 |
| | | U6 | 2.10 | 0.86 | 1.80 | 0.34 | 2.40 | 1.53 | |
| | | U7 | 1.80 | 0.74 | 2.10 | 0.40 | 3.10 | 1.97 | |
| | | U8 | 1.70 | 0.70 | 2.00 | 0.38 | 1.40 | 0.89 | |
| | | U9 | 52.70 | 21.59 | 45.70 | 8.64 | 44.50 | 28.33 | GR43175 |

MALE

Quantitative assessment by [illegible] of radioactivity present in urine following a single intravenous administration of [illegible] to male dogs at a nominal dose level of 1 mg/kg body weight

| Sample | Time point | Region | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1M | 1M | 2M | 2M | 3M | 3M | |
| Urine | 6 to 24 h | U1 | 2.90 | 0.88 | 1.80 | 0.28 | 2.80 | 0.29 | |
| | | U2 | 3.90 | 1.19 | 3.40 | 0.54 | 7.70 | 0.81 | |
| | | U3 | 1.80 | 0.55 | 2.80 | 0.44 | 4.60 | 0.48 | |
| | | U4 | 0.80 | 0.24 | 0.70 | 0.11 | 0.20 | 0.02 | |
| | | U5 | 46.70 | 14.23 | 42.90 | 6.79 | 45.40 | 4.75 | GR49336 |
| | | U6 | 3.20 | 0.98 | 2.50 | 0.40 | 4.00 | 0.42 | |
| | | U7 | 4.00 | 1.22 | 4.10 | 0.65 | 5.80 | 0.61 | |
| | | U8 | 1.40 | 0.43 | 3.10 | 0.49 | 1.80 | 0.19 | |
| | | U9 | 35.20 | 10.71 | 38.50 | 6.09 | 27.00 | 2.83 | GR43175 |

FEMALE

Quantitative assessment by [illegible] of radioactivity present in urine following a single intravenous administration of ( to female dogs at a nominal dose level of 1 mg/kg body weight

| Sample | Time point | Region | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|
| | | | 4F | 4F | 5F | 5F | 6F | 6F | |
| Urine | 0 to 6 h | U1 | ND | ND | 2.40 | 0.77 | ND | ND | |
| | | U2 | ND | ND | 0.60 | 0.19 | ND | ND | |
| | | U3 | ND | ND | 1.70 | 0.55 | ND | ND | |
| | | U4 | ND | ND | 5.50 | 1.77 | ND | ND | |
| | | U5 | 49.10 | 0.02 | 26.10 | 8.41 | ND | ND | GR49336 |
| | | U6 | ND | ND | 2.40 | 0.77 | ND | ND | |
| | | U7 | ND | ND | 1.90 | 0.61 | ND | ND | |
| | | U8 | ND | ND | 1.40 | 0.45 | ND | ND | |
| | | U9 | 50.90 | 0.02 | 57.80 | 18.63 | ND | ND | GR43175 |

FEMALE

Quantitative assessment b[illegible] of radioactivity present in urine following a single intravenous administration of[illegible] to female dogs at a nominal dose level of 1 mg/kg body weight

| Sample | Time point | Region | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|
| | | | 4F | 4F | 5F | 5F | 6F | 6F | |
| Urine | 6 to 24 h | U1 | 1.7 | 1.27 | 1.3 | 0.54 | 3.0 | 0.74 | |
| | | U2 | 3.1 | 2.32 | 2.2 | 0.91 | 3.3 | 0.81 | |
| | | U3 | 2.1 | 1.57 | 1.7 | 0.70 | 3.5 | 0.86 | |
| | | U4 | 0.9 | 0.67 | 0.7 | 0.29 | 0.9 | 0.22 | |
| | | U5 | 45.0 | 33.66 | 48.6 | 20.01 | 41.3 | 10.14 | GR49336 |
| | | U6 | 2.2 | 1.65 | 1.8 | 0.74 | 2.6 | 0.64 | |
| | | U7 | 2.5 | 1.87 | 3.5 | 1.44 | 4.2 | 1.03 | |
| | | U8 | 2.4 | 1.80 | 1.9 | 0.78 | 2.1 | 0.52 | |
| | | U9 | 39.9 | 29.85 | 37.6 | 15.48 | 39.0 | 9.57 | GR43175 |

Quantitative assessment by of radioactivity present in urine following a single intranasal administration of to male dogs at a nominal dose level of 1 mg/kg body weight

| Sample | Time point | Region | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1M | 1M | 2M | 2M | 3M | 3M | |
| Urine | 0 to 6 h | U1 | 3.2 | 1.18 | 2.5 | 1.25 | 3.8 | 1.65 | |
| | | U2 | 1.7 | 0.63 | 2.5 | 1.25 | 1.7 | 0.74 | |
| | | U3 | 0.7 | 0.26 | 1.3 | 0.65 | 0.2 | 0.09 | |
| | | U4 | 40.6 | 14.99 | 38.3 | 19.19 | 48.5 | 21.01 | |
| | | U5 | 11.2 | 4.14 | 18.9 | 9.47 | 10.7 | 4.63 | GR49336 |
| | | U6 | 1.4 | 0.52 | 1.5 | 0.75 | 1.2 | 0.52 | |
| | | U7 | 1.6 | 0.59 | 2.1 | 1.05 | 1.9 | 0.82 | |
| | | U8 | 2.1 | 0.78 | 1.4 | 0.70 | 1.3 | 0.56 | |
| | | U9 | 37.0 | 13.66 | 30.8 | 15.43 | 29.9 | 12.95 | GR43175 |

Quantitative assessment by of radioactivity present in urine following a single intranasal administration of o male dogs at a nominal dose level of 1 mg/kg body weight

| Sample | Time point | Region | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1M | 1M | 2M | 2M | 3M | 3M | |
| Urine | 6 to 24 h | U1 | 2.70 | 0.57 | 2.50 | 0.46 | 3.20 | 0.63 | |
| | | U2 | 4.60 | 0.96 | 4.80 | 0.88 | 5.00 | 0.98 | |
| | | U3 | 3.00 | 0.63 | 3.50 | 0.64 | 3.40 | 0.67 | |
| | | U4 | 0.60 | 0.13 | 1.20 | 0.22 | 1.60 | 0.31 | |
| | | U5 | 45.00 | 9.42 | 47.90 | 8.77 | 44.70 | 8.75 | GR49336 |
| | | U6 | 2.60 | 0.54 | 2.20 | 0.40 | 2.80 | 0.55 | |
| | | U7 | 3.40 | 0.71 | 4.30 | 0.79 | 5.20 | 1.02 | |
| | | U8 | 2.00 | 0.42 | 1.80 | 0.33 | 1.50 | 0.29 | |
| | | U9 | 35.30 | 7.39 | 30.80 | 5.64 | 32.50 | 6.36 | GR43175 |

Quantitative assessment by of radioactivity present in urine following a single intranasal administration of to female dogs at a nominal dose level of 1 mg/kg body weight

| Sample | Time point | Region | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|
| | | | 4F | 4F | 5F | 5F | 6F | 6F | |
| Urine | 0 to 6 h | U1 | 2.5 | 1.04 | ND | ND | ND | ND | |
| | | U2 | 1.9 | 0.79 | ND | ND | ND | ND | |
| | | U3 | 1.6 | 0.67 | ND | ND | ND | ND | |
| | | U4 | 0.8 | 0.33 | ND | ND | ND | ND | |
| | | U5 | 51.2 | 21.30 | ND | ND | ND | ND | GR49336 |
| | | U6 | 2.5 | 1.04 | ND | ND | ND | ND | |
| | | U7 | 2.1 | 0.87 | ND | ND | ND | ND | |
| | | U8 | 1.6 | 0.67 | ND | ND | ND | ND | |
| | | U9 | 35.1 | 14.60 | ND | ND | ND | ND | GR43175 |

Quantitative assessment by of radioactivity present in urine following a single intranasal administration of to female dogs at a nominal dose level of 1 mg/kg body weight

| Sample | Time point | Region | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Per Cent Radioactivity in region | Per Cent dose in peak | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|
| | | | 4F | 4F | 5F | 5F | 6F | 6F | |
| Urine | 6 to 24 h | U1 | 1.10 | 0.23 | 2.40 | 1.53 | 2.70 | 1.75 | |
| | | U2 | 4.40 | 0.91 | 2.20 | 1.41 | 3.20 | 2.07 | |
| | | U3 | 3.20 | 0.66 | 1.70 | 1.09 | 2.10 | 1.36 | |
| | | U4 | 1.00 | 0.21 | 0.90 | 0.58 | 13.10 | 8.47 | |
| | | U5 | 46.70 | 9.64 | 49.10 | 31.38 | 38.00 | 24.56 | GR49336 |
| | | U6 | 1.70 | 0.35 | 2.40 | 1.53 | 2.30 | 1.49 | |
| | | U7 | 3.60 | 0.74 | 2.40 | 1.53 | 3.20 | 2.07 | |
| | | U8 | 1.80 | 0.37 | 1.60 | 1.02 | 1.60 | 1.03 | |

**in Phosphate Buffered Solution: A 14 Day Intranasal Irritancy Study in the Dog**

Testing Facilities: #1 Glaxo Group Research Ltd., UK (in-life & necropsy) (GLP).
#2

#3
#4

Dates of Dosing: 1/14/93 - 1/29/93

Test Material: The test formulation was equivalent to the proposed clinical formulation. the hemisulfate salt of ( was supplied as autoclaved vials of a solution of 200 mg(base)/ml in phosphate buffer. Vials of autoclaved phosphate buffer served as the vehicle control. The batches were as follows (with stated storage conditions: 21-24 °C, in the dark, and expiry dates of 6/10/93 and 6/3/93 respectively):
drug: batch # F92/184D, Toxicology reference # TY1/24
vehicle control: batch # F92/183D, Toxicology reference # TY1/23

Stability: The sponsor analyzed the solution prior to the initiation of the study (12/15/92) and after the last dosing (2/17/93) and found that the pH had decreased from 5.6 to 5.4. There were no apparent changes in the amount of the parent compound or the impurities (within the limits of the sponsor's methodology).

Analysis: Analysis of the dosing solution was carried out by UV spectroscopy on days 1 and 14 and the mean values were found to be 204.7 and 203.0 mg(base)/ml compared to the nominal concentration of 200 mg(base)/ml.

Animal: Beagle dogs from Glaxo's own colony, 3-4 months old at initiation of dosing and weighing 5.47-7.16 kg for males and 5.36 - 6.60 kg for females were used. Animals were housed in groups of two (same sex and group), with free access to food and water. Animals were assigned randomly to treatment groups, with manipulation to ensure an even distribution of litter mates among groups.

Route: Intranasal from a metered dose pump (Valois). The solutions in the pump were changed daily. During dosing, animals were restrained with the head tilted slightly upward.

Dosing Rationale: According to the sponsor 12 sprays/day (3 sprays/nostril, b.i.d.) was the maximum achievable dose volume as determined in the preliminary study D12278 (report WPT/90/325) (with a different formulation). For the current study, the adverse reaction to treatment during the first dosing session of day one resulted in a decision to decrease the daily dose to six sprays/day as three sprays to the right nostril, b.i.d.

| DOSING REGIMEN | | | | | |
|---|---|---|---|---|---|
| group | treatment | dose [mg(base)] | concentration [mg(base)/ml] | volume [ml] | #/sex |
| control | phosphate buffer | originally planned: 0, b.i.d.<br>after protocol alteration: 0, b.i.d. | ~~200~~ 0 | originally planned: 0.6, b.i.d.<br>after protocol alteration: 0.3, b.i.d. | 4 |
| treated | GR43175N in buffer | originally planned: 120, b.i.d.<br>after protocol alteration: 60, b.i.d. | 200 | originally planned: 0.6, b.i.d.<br>after protocol alteration: 0.3, b.i.d. | 4 |

Two dosing sessions per day were planned for each animal. Because of severe clinical signs after the 1st dose on day one, only one dosing session was carried out. For subsequent days, the dose was reduced, such the individual dose that each session was halved.

There was a change in dosing regimen :

day 1 3 sprays per nostril, b.i.d. - total = 240 mg(base)/dog/day. Dosing occurred only once due to adverse reactions
day 2 -14/15 3 sprays to right nostril only, b.i.d. - total = 120 mg(base)/dog/day

There were two dosing sessions per day. The time period between the first and second dosings varied between 3 hr and 20 min and 5 hrs and 20 min.

Each spray was 0.1 ml; therefore, from day 2 through the end of the study the animals received 0.6 ml/day.

| PROTOCOL | | |
|---|---|---|
| parameter | animals tested | description |
| treatment | all | once on day 1, then twice per day from day 2 for 13/14 consecutive days |
| physical exam | all | pretreatment period and day 13 |
| clinical signs | all | at least six times daily |
| body weight | all | pretreatment and days 1, 7, 14 and prior to autopsy day 15/16 |
| food consumption | all/kennel<br>2/kennel | daily for one week prior to initiation of treatment and during weeks 1 and 2 of dosing |
| plasma drug concentration | all | days 1/2 and days 13/14 - from jugular<br>day 1: predose, 1 hr post dose<br>day 2: 24 hrs after day one dose, 1 hr post 1st dose, 1 hr post 2nd dose<br>day 13: predose, 1 hr post 1st dose, 1 hr post 2nd dose<br>day 14: 24 hrs after the 2nd dose of day 13 |
| post mortem studies | all | anesthetized with iv pentobarbitone and exsanguinated on day 15/16 (the day after the last dose)<br>full macroscopic exam on each animal |
| histopathology | all | READ UNBLINDED<br>full compliment of tissues retained; however, only the following tissues were examined: trachea, lungs, larynx and oropharynx (epiglottis/pharynx), tracheobronchial lymph node, nasal passages<br>fixed in 10% buffered formalin, paraffin embedded, H&E, LM only |

**Results**

Mortality: none

Clinical Signs:

1. There were no clinical signs in the vehicle control group.

2. On day 1, there was only one dosing session instead of the scheduled two per day. After the administration of the first dose of (3 sprays per nostril for a total of 120 mg(base)/dog) there were clinical signs described by the sponsor as severe (pupil dilation, trembling, splayed hindlimbs, unsteady gait, vocalization and subdued behavior). Many of these signs were still present 8 hours after the initial dose. Therefore, the sponsor chose to not to administer the second treatment on day 1 and to halve the dose from day 2 through the end of the study (3 sprays to the right nostril, b.i.d.).

3. In general clinical signs consisted of: salivation, subdued behavior, pupil dilation, trembling, splayed hindlimbs, vocalizing, unsteadiness, head shaking, aggressive behavior, nose running, lip licking, and stiff hindlimbs. From day 2 onward there were two dosing sessions per day. Although there are no specific predose notations for the first dosing of each day, the sponsor states that the treatment related clinical signs had regressed by the next morning. Salivation and head shaking were noted immediately after dosing (first and second dose of the day) in addition, on several occasions pupil dilation, splayed hindlimbs, vocalization and unsteadiness were noted immediately after the second dose of the day. On the majority of treatment days clinical signs were noted prior to the administration of the second dose. These usually consisted of dilated pupils, splayed hindlimbs, unsteadiness, vocalization and occasionally trembling and stiff hindlegs. At approximately one hour after dosing, pupil dilation, splayed hindlimbs, vocalizing were generally noted with occasional unsteadiness and aggressive behavior. At approximately 4-5 hours after dosing, pupil dilation, splayed hindlimbs, vocalizing were generally noted with occasional notations of unsteadiness and trembling.

Body Weight: Three of the four treated males and one of four treated females showed reductions in body weight between days 1 and 7 of treatment; however, these animals showed increases in body weight between days 7 and 14.

| Mean change in body weight per group | | | |
|---|---|---|---|
| group | days 1-7 | days 1-15 | days 1-16 |
| control | males - 0.38 ± 0.19<br>females - 0.17 ± 0.12 | males - 0.71 ± 0.15<br>females - not determined | males - not determined<br>females - 0.47 ± 0.03 |
| GR43175 treated | males - 0.005 ± 0.15<br>females - 0.05 ± 0.27 | males - 0.30 ± 0.20<br>females - not determined | males - not determined<br>females - 0.48 ± 0.34 |

Food Consumption: Recall that this parameter was determined by kennel and that each kennel contained two animals of the same sex and treatment group. The data presented is very variable. This data suggests that there was adequate food consumption for each kennel grouping. Nothing can be said about an individual animal. It appears that the per kennel consumption was decreased at several different points in the study for the treated groups. More definitive statements cannot be made. The sponsor notes that on the first two days of treatment, food consumption in the treated groups was lower than anticipated.

Plasma Drug Concentration: According to the sponsor, detectable plasma levels of parent compound were seen in all ( treated animals on days 1, 2 and 13 this indicating that there was exposure to the drug. Plasma levels were variable. While plasma levels were detectible 24 hours post dose in some animals, there was no evidence of accumulation. A reviewer-generated summary table follows.

Plasma Concentration (ng(base)/ml)

| day/time | males | | females | |
|---|---|---|---|---|
| | mean ± S.D. | range | mean ± S.D. | range |
| day 1 - predose | < 10 * | | < 10 * | |
| day 1 - 1 hr post dose | 2558 ± 1214 | | 3383 ± 1576 | |
| day 1 - 24 hrs post dose<br>day 2 - predose | - | | < 10 * | |
| day 2 - 1 hr post 1st dose | 502 ± 289 | | 856 ± 774 | |
| day 2 - 1 hr post 2nd dose | 513 ± 217 | | 568 ± 403 | |
| day 13 - predose | 14.4 ± 5.8 | | 25.4 ± 7.6 | |
| day 13 - 1 hr post 1st dose | 1219 ± 293 | | 908 ± 409 | |
| day 13 - 1 hr post 2nd dose | 767 ± 276 | | 895 ± 345 | |
| day 13 - 24 hr post 2nd dose | 10.5 ± 1.9 | | 11.2 ± 3.7 | |

HPLC with electrochemical detection (limits of detection (10 - 500 ng(base)/ml)

* on this occasion the lower limit of detection was 10 ng/ml

Note that on day 13 there were quantifiable levels of parent compound (range, 5.0-16.1 ng(base)/ml) in 6 of the 8 control animals, which according to the sponsor's report, was due to interfering endogenous plasma component.

Necropsy: Necropsy was carried out by the sponsor. According to the sponsor, there were no treatment related findings. Examination of the data demonstrates one treated male was noted with a firm reddened area of the lung (noted with bronchopneumonia/pneumonitis and congestion/hemorrhage on histopathology), and two treated males were noted with red tracheobronchial lymph nodes (noted with congestion/hemorrhage on histopathology).

Histopathology: Tissue processing was carried out by Histopathology was carried out at by a contract group. According to the pathologist there were no treatment related findings: "A variety of very minor degenerative and inflammatory lesions were observed. These fell within the range for dogs of this age and strain, and were considered unrelated to treatment."

Examination of the data reveals that one of the eight ( treated animals (female) was noted with focal squamous metaplasia of the bronchial epithelium of the lungs (grade 1). A copy of the sponsor-supplied summary table follows.

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 01, CONTROL

| ANIMAL NUMBER : | 5 9266 1K0 | 5 9268 1K0 | 5 9270 1K0 | 5 9272 1K0 | 5 9274 2K0 | 5 9276 2K0 | 5 9278 2K0 | 5 9280 2K0 |
|---|---|---|---|---|---|---|---|---|
| GENERAL OBSERVATIONS | ' | ' | ' | ' | ' | ' | ' | ' |
| OROPHARYNX | - | - | - | - | - | - | - | - |
| LARYNX | . | . | . | . | . | . | . | . |
| - ACUTE INFLAMMATION | . | . | . | . | 1* | . | . | . |
| - LYMPHOID FOCI | 1. | 1. | 2. | 1. | . | 1. | 1. | 1. |
| TRACHEA | - | - | - | . | - | - | - | - |
| - INTRA-EP PMN INFILT | . | . | . | 1. | . | . | . | . |
| LUNGS | . | . | . | . | . | . | . | . |
| - PNEUMONIA/ITIS | 1. | 1. | 1. | 1. | 1. | 1. | 1. | 1. |
| - FOCAL ALVEOL MACROS | . | 1. | 1. | . | 1. | . | . | . |
| TRACHEOBRON LYMPH N | . | . | . | . | . | . | . | . |
| - CONGESTION/HAEM | 1. | 1. | 1. | 1. | 1. | 2. | 1. | 1. |
| - ACUTE INFLAMMATION | . | . | 1. | 1. | . | 1. | 1. | 1. |
| NASAL CAVITY, ANTER. | . | - | - | - | - | - | - | . |
| - ACUTE INFL EXUDATE | 1. | . | . | . | . | . | . | 1. |
| NASAL CAVITY, POST. | - | - | - | - | - | - | - | - |
| THYROID GLANDS | 'G | ' | ' | ' | ' | ' | ' | ' |
| TESTES | ' | ' | ' | 'G | | | | |
| SPLEEN | ' | ' | ' | ' | ' | ' | ' | 'G |

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 02, 120 MG/DAY

| ANIMAL NUMBER : | 5 9267 1K0 | 5 9269 1K0 | 5 9271 1K0 | 5 9273 1K0 | 5 9275 2K0 | 5 9277 2K0 | 5 9279 2K0 | 5 9281 2K0 |
|---|---|---|---|---|---|---|---|---|
| GENERAL OBSERVATIONS | ' | ' | ' | ' | ' | ' | ' | ' |
| OROPHARYNX | - | - | - | - | - | - | - | - |
| LARYNX | . | . | - | . | . | . | . | . |
| - LYMPHOID FOCI | 1. | 1. | . | 1. | 1. | 1. | 1. | 2. |
| TRACHEA | - | . | - | - | - | - | - | - |
| - INTRA-EP PMN INFILT | . | 1. | . | . | . | . | . | . |
| LUNGS | . | G | . | . | . | - | . | . |
| - PNEUMONIA/ITIS | 1. | 2* | 1. | 1. | 1. | . | 1. | 1. |
| - FOCAL ALVEOL MACROS | . | . | 1. | . | 1. | . | . | . |
| - CONGESTION/HAEM | . | 2* | . | . | . | . | . | . |
| - FOCAL SQUAMOUS MET | . | . | . | . | 1. | . | . | . |
| TRACHEOBRON LYMPH N | . | G | . | G | . | . | - | - |
| - CONGESTION/HAEM | 1. | 2* | 1. | 2* | . | 1. | . | . |
| - ACUTE INFLAMMATION | . | 1. | 2. | 1. | 1. | 1. | . | . |
| NASAL CAVITY, ANTER. | - | - | - | - | - | - | - | - |
| NASAL CAVITY, POST. | - | . | - | - | - | - | - | - |
| - ACUTE INFL CELL FOCI | . | 1. | . | . | . | . | . | . |
| THYROID GLANDS | ' | ' | ' | ' | ' | ' | ' | ' |
| TESTES | ' | ' | ' | ' | | | | |
| SPLEEN | ' | ' | ' | ' | ' | ' | ' | ' |

CODES AND SYMBOLS USED AT ANIMAL LEVEL

1 = MALE
2 = FEMALE
K0 = TERMINAL SACRIFICE GROUP

CODES AND SYMBOLS USED AT ORGAN LEVEL:

G = GROSS FINDING EVALUATED HISTOLOGICALLY
' = HISTOLOGIC EXAMINATION NOT REQUIRED
- = ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

CODES AND SYMBOLS USED AT FINDING LEVEL:

GRADE 1 = MINIMAL / VERY FEW / VERY SMALL
GRADE 2 = SLIGHT / FEW / SMALL
* = COMMENT IN TEXT OF INDIVIDUAL ANIMAL DATA

**Nasal Spray: Acute Eye Irritation Study in the Rabbit**

Testing Facility: (1)

(2) Glaxo Research and Development Ltd.
includes a GLP statement

Study Date: 12/14/94 - 12/23/94

Test Material: This study used the proposed clinical formulation. Nasal Spray, 200 mg/ml, buffered, (batch # F632/2) was supplied by the sponsor, with the notation to store at room temperature, protected from light. Test material was used as supplied. According to the report the pH was approximately 6.0.

Stability: Historic stability information was supplied to support a two year shelf life. Stability under the specific conditions of the assay were not assessed.

Animals: New Zealand White Rabbits (one male and three female) from ., 12 -16 weeks old and 2.60 - 3.08 kg at the initiation of the study were used. The animals were housed individually and had free access to food and water. One day prior to treatment, both eyes from each animal were examined under UV light after treatment with fluorescein and the cornea, iris and conjunctiva were examined. The eyes were examined again, just prior to treatment, using an ophthalmoscope. There is a notation that only those animals with no ocular damage were used.

Protocol: A stated volume of the test formulation was placed in the conjunctival sac of the right eye of a rabbit. Following administration the eyelids were held together for approximately one second. Immediately afterward the eye was assessed for the initial local pain reaction using the following rating scale. The left, untreated eye served as a control.

| Initial Pain Reaction | | |
|---|---|---|
| class | reaction by animal | descriptive rating |
| 0 | no response | no initial pain |
| 1 | a few blinks only, normal within one or two minutes | practically no initial pain |
| 2 | rabbit blinks & tries to open eye, but reflex closes it | slight initial pain |
| 3 | rabbit holds eye shut & puts pressure on lids, may rub eye with paw | moderate initial pain |
| 4 | rabbit holds eye shut vigorously, may squeal | severe initial pain |
| 5 | rabbit holds eye shut vigorously, may squeal, claw at eye, jump & try to escape | very severe initial pain |

Assessments of damage or irritation to the eye were made at 1, 3, 6, 24, 48 and 72 hours post treatment, using the light from a standard ophthalmoscope. At the 24 hour examination, the cornea was examined for presence or absence of opacity under UV light with fluorescein. This was repeated at later time points if an opacity were noted. The sponsor-provided scales used for evaluation follow. Treatment groups were as follows:

(1) one rabbit using 0.01 ml of the test formulation.
(2) one rabbit using 0.1 ml of the test formulation.
(3) two additional rabbits using 0.1 ml of the test formulation.

| Rating System | | |
|---|---|---|
| part of the eye | designation | observation |
| conjunctiva | A | redness |
| | B | chemosis |
| | C | discharge |
| iris | D | unspecified |
| cornea | E | degree of opacity |
| | F | area of opacity |

Scoring System

Score for conjunctiva = (A + B + C) x 2
Score for iris = D x 5
Score for cornea = (E x F) x 5

the mean score for each part of the eye was obtained, as well as a mean total score (score for iris + conjunctiva + cornea)

| Classification of ocular irritancy potential | | |
|---|---|---|
| grade | classification | maximum overall mean score |
| $0^x$ | non-irritant | 0 - 4 |
| $1^x$ | negligible risk of eye damage | 5 - 11 |
| 2 | slight irritant | 12 - 15 |
| 3 | moderately irritant | 16 - 40 |
| 4 | severe risk of eye damage | 41 - 65 |
| 5 | very sever risk of eye damage | 66 - 110 |

$^x$ - reaction must be limited to the conjunctiva only for grades 0 or 1

test article will be labeled as corrosive to eye if there is evidence of irreversible ocular damage

| Interpretation of Results | | |
|---|---|---|
| part of eye | mean score | interpretation |
| conjunctiva | 1 - 5 | very slight reaction |
| | 6 - 8 | slight reaction |
| | 9 - 11 | mild reaction |
| | 12 - 16 | moderate reaction |
| | 17 - 20 | marked reaction |
| iris | 1 - 2 | mild reaction |
| | 3 - 7 | moderate reaction |
| | 8 - 10 | marked reaction |
| cornea | 1 - 10 | slight reaction |
| | 11 - 40 | moderate reaction |
| | 41 - 75 | marked reaction |
| | 76 - 80 | very marked reaction |

Results

Initial Pain Reaction: The low dose (0.01 ml) animal was noted with a score of 1: no initial pain. Each of the three animals in the high dose (0.1 ml) group were noted with scores of 2: slight initial pain.

Irritation to the Conjunctiva: The low dose (0.01 ml) animal was noted only with redness at one hour after dose; therefore, the total score was 2 and the mean conjunctival score at this dose was 2. For the high dose (0.1 ml) animals, two of the three animals were noted only with redness one hour after dosing. Therefore, the total scores were 2, 2 and 0, and the mean total score was 1.3. According to the classification system used, for both treatment groups, this was considered a very slight reaction.

Irritation to the Iris: No animal in either dose group (0.01 ml or 0.1 ml) had any indication of irritation or damage.

Irritation to the Cornea: No animal in either dose group (0.01 ml or 0.1 ml) had any indication of irritation or damage.

Overall Classification: For both the low dose group (0.01 ml) and the high dose group (0.1 ml) the test formulation (200 mg/ml- buffered) would be considered a non-irritant.

**13 Week Intranasal Tolerance Study in Dogs**

Testing Facility:

Dates of Dosing: 5/23/91 - 9/23/91

Test Material: Note that this formulation is different from the clinical formulation. The formultion used in this study is unbuffered, whereas the clinical formulation is buffered. Dosing materials were supplied by the sponsor in sealed glass vials.

The placebo (batch # F91/084A) consisted of purified water and sodium hydroxide or sulfuric acid. Two strengths o the hemi-sulfate of the base ( were supplied - 200 mg/ml - batch # F91/085A and ( - 400 mg/ml - batch # F91/086A. The is formed *in situ* by a reaction of the base and sulfuric acid and sodium hydroxide in purified water. The test formulations were marked with an 11/91 expiry date. It was noted that when not in use, the vials should be stored at 19-27 °C, protected from light.

Stability: Evidence of stability of the test formulations under the condition of the study were not included in the report. The sponsor provides historical information on the stability of the formulations stored for up to 7 months at either 2 °C or 30 °C. Note that under these circumstances there is a downward shift in pH (by approximately one half a unit) for the formulations and a slight increase in the amount of impurities. According to the sponsor the slight changes noted in the amount of impurities were not significant.

Concentration Analysis: The test formulation was noted to be within 1% of the nominal value.

Animal: Pure bred Beagle dogs from were used in this study. At the initiation of treatment animals were 4-6 months old and the males weighed 6.80 - 10.00 kg and the females weighed 5.95 - 7.95 kg. Animals were housed singly during the day (when food was present) and in pairs (by sex and group) at night (after food removal). Food was provided as 400 grams per day, and the animals had free access to water. The dogs were randomly assigned to treatment group using stratified body weight and, whenever possible, litter mates were distributed across groups.

Route: Intranasal, using a metered pump dosing device. The type of device was not named. One dosing device was used for each group and according to the sponsor, there was a sufficient volume in each vial to treat all animals in that group on a particular day. The containers of the dosing device were weighed after each dosing session. Prior to the initiation of the test, the volumes of delivery for test and control solutions were determined, and found to be 92 - 114 mg, compared to the nominal 100 mg.

All animals were familiarized with the dosing procedure through two days of sham dosing, carried out during the final three days of acclimation. According to the sponsor: "Dosing started with the left nostril during inspiration and with the external nares elevated." The sponsor notes that from day 7 onward the eyes of all animals were covered, on veterinary advice, based on a notation of corneal opacity in the eyes of several animals on day 6.

Dosing Rationale: According to the sponsor, the concentrations of the dosing solutions and the volume of each puff, were chosen because they were to be employed in the clinical situation. The sponsor also points out that in study # D12278, the maximum practicable intranasal dose was found to be 240 mg base/twice daily.

DOSING REGIMEN

| group | treatment | dose mg(base) | concentration mg(base)/ml | # sprays | volume | #/sex |
|---|---|---|---|---|---|---|
| control | vehicle | - | 0 | 3/nostril/session<br>total=6 sprays/session | 0.1 ml/spray<br>total=0.6 ml/session | 4 |
| | | | | 6/nostril/day<br>total = 12 sprays/day | total = 1.2 ml/day | |
| low dose | GR43175N | 120/session<br>total = 240/day | 200 | 3/nostril/session<br>total=6 sprays/session | 0.1 ml/spray<br>total=0.6 ml/session | 4 |
| | | | | 6/nostril/day<br>total = 12 sprays/day | total = 1.2 ml/day | |
| high dose | GR43175N | 240/session<br>total = 480/day | 400 | 3/nostril/session<br>total=6 sprays/session | 0.1 ml/spray<br>total=0.6 ml/session | 4 |
| | | | | 6/nostril/day<br>total = 12 sprays/day | total = 1.2 ml/day | |

Total daily dosage was divided over two daily sessions, separated by approximately 4 - 6 hrs, except day 1 when only one dose was administered to allow for PK studies. Twice daily doses were carried out from day 2-89/90, excluding the day of necropsy.

PROTOCOL

| parameter | animals tested | description |
|---|---|---|
| drug administration | all | Once on day 1; twice daily from day 2-89/90, excluding day of necropsy. |
| clinical signs | all | Monitored in morning prior to feeding and towards the end of the working day. On day 2 animals were observed hourly. From day 3 on animals were checked throughout the working day as needed to monitor progress of clinical signs. |
| eyes covered | all | From day 7 onward eyes were covered during dosing due to the appearance of superficial corneal opacity noticed in several treated animals by the veterinarian on day 6. (no notations about reversibility (#2329F, 2331F, 2333F). |
| body weights | all | Weekly. |
| food consumption | all | Recorded daily, calculated weekly. |
| plasma drug concentrations | all | day 1: prior to dosing, 5 min, 30 min, 1, 2, 4, 6, 8 24 hrs post dose.<br>day 35: prior to 1st dose, 5 min, 30 min, 1, 2, 4, hrs post 1st dose and 1, 2, 4, 6, 8, and 10 hrs post 2nd dose (second dose was just after the 4 hr sampling after the 1st dose).<br>week 13: once prior to the 1st dose and at 1 hr after the 2nd dose (2nd dose administered 4 hrs after the 1st dose).<br>by HPLC with electrochemical detection. |
| necropsy | all | Carried out over the course of two days. Animals were given intravenous injection of sodium thiopentone following an overnight fast, then exsanguinated, followed by full internal and external examination. A similar number of males and females from each group were processed per day. |
| organ weights | all | Adrenals, brain + brain stem, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes and epididymis, thyroid and parathyroids. As absolute weights only. |
| histopathology | all | A full compliment of tissues were sampled; however, only the following tissues were examined: larynx with pharynx, lung with mainstem bronchi, retropharyngeal and bronchial lymph nodes, nasal passages, tongue, trachea and bifurcation, and all gross lesions.<br>immersion fixed in 10% neutral buffered formalin, paraffin embedded, H & E stained. |

Results

Mortality: none

Clinical Signs: One control female had a single incidence of salivation; otherwise, there were no clinical observations in the control group. Treatment related salivation and mydriasis were noted in all of the treated animals, throughout the study. According to the sponsor salivation was immediate and lasted 1-5 minutes and mydriasis was noted 1-2 hours after the first daily dosing and still noted 4 hours after the second daily dosing. According to the sponsor mydriasis was usually gone before the first dose of the next day and the frequency of salivation was reduced as the study progressed. On day 6 superficial corneal opacities were noted in three treated females, one from the low dose group (# 2329) and two from the high dose group (#2331 and #2333). This issue is not discussed anywhere and there were no notations of follow-up.

Body weight: There were no obvious no adverse effect of treatment on body weight or body weight gain over the course of the study.

| Mean (± S.D.) Body Weight Gain from Week -1 through 13 | | |
|---|---|---|
| control | males - 2.08 ± 0.96 | females - 1.49 ± 0.09 |
| low dose | males - 2.39 ± 0.12 | females - 1.90 ± 0.40 |
| high dose | males - 2.39 ± 0.57 | females - 1.14 ± 0.57 |

Food Consumption: No pretreatment values were recorded for the two weeks prior to treatment; however, there were no obvious effects of treatment.

Necropsy: According to the sponsor: "Necropsy findings in most tissues and organs were unremarkable... Minor findings included red areas and foci in various parts of the alimentary tract and discoloration of the lungs." The reviewer agrees.

Organ Weights: Organ weights were recorded only as absolute organ weights. According to the sponsor, there was no evidence of an effect of treatment on organ weight. The sponsor points out that there was a statistically significant increase in mean brain weight of high dose females (8%); however, the sponsor states that the isolated incidence and magnitude of the change make this finding of no toxicological consequence. The reviewer notes isolated increases and decreases with no clear pattern, and generally in organs on which pathology was not carried out. With such small groups for comparison these changes in isolation are not meaningful.

Histopathology: Copies of the sponsor-supplied summary table follows. According to the sponsor: "There were no significant findings in the nasal cavities or other parts of the upper respiratory tract." The sponsor did point out the various types of inflammatory lesions seen in the lungs are those which are regularly encountered in this age and strain of dogs and, therefore, are not treatment related. According to the sponsor there were no findings suggestive of any affect of treatment.

Based on the small size of the treatment groups it is probably best to combine males and females for an additional data analysis. A reviewer-generated table of possibly noteworthy changes follows.

| Histopathology | | | |
|---|---|---|---|
| | control | low dose | high dose |
| lung - fibrosing alveolitis | 1/8 | 1/8 | 3/8 |
| lung - bronchitis | 0/8 | 2/8 | 2/8 |
| lung - granuloma | 0/8 | 0/8 | 2/8 |
| larynx - lymphoid hyperplasia (minimal) | 0/8 | 0/8 | 1/8 |
| bronchial lymph node - hyperplasia (slight) | 0/8 | 1/8 | 0/8 |
| nasal passages - lymphoid hyperplasia (minimal) (respiratory region) | 0/8 | 0/8 | 1/8 |
| stomach - lymphoid hyperplasia (minimal) | 0/8 | 1/8 | 1/8 |
| skin - dermatitis | 0/8 | 1/8 | 2/8 |

Plasma Drug Concentration: Copies of the sponsor-supplied summary tables follow. According to the sponsor there were no sex differences so they analyzed the data in of both sexes combined. The sponsor notes that drug was detected in control samples; in low quantities compared to the treatment groups. Note that 24 hours after the only dose on day 1 there was still detectable plasma concentrations. Somewhat higher values were noted predose on day 35; however, there were two dosing session on day 34.

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ON ORIGINAL

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TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

| ORGAN AND FINDING DESCRIPTION | | --- NUMBER-OF-ANIMALS-AFFECTED --- | | | | | |
|---|---|---|---|---|---|---|---|
| SEX: | | -----MALE---- | | | ----FEMALE--- | | |
| GROUP: | | -1- | -2- | -3- | -1- | -2- | -3- |
| NUMBER: | | 4 | 4 | 4 | 4 | 4 | 4 |
| ** TOP OF LIST ** | | | | | | | |
| LUNG | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --EXTRA SAMPLE | | 1 | 0 | 0 | 0 | 0 | 2 |
| --LEUCOCYTE FOCI | | 4 | 2 | 4 | 4 | 4 | 3 |
| --PNEUMONITIS | | 2 | 1 | 1 | 1 | 0 | 0 |
| --PIGMENTED HISTIOCYTES | | 1 | 0 | 0 | 0 | 0 | 0 |
| --FIBROSING ALVEOLITIS | | 1 | 1 | 2 | 0 | 0 | 1 |
| --BRONCHITIS | | 0 | 2 | 1 | 0 | 0 | 1 |
| --GRANULOMA | | 0 | 0 | 1 | 0 | 0 | 1 |
| --ECTOPIC BONE | | 0 | 0 | 0 | 0 | 0 | 1 |
| LARYNX | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --LYMPHOID HYPERPLASIA | | 0 | 0 | 1 | 0 | 0 | 0 |
| BRONCHIAL LN | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --ERYTHROPHAGOCYTOSIS | | 0 | 1 | 0 | 0 | 0 | 0 |
| --HYPERPLASIA | | 0 | 1 | 0 | 0 | 0 | 0 |
| NASAL PASSAGES | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --HAEMORRHAGE | | 0 | 1 | 0 | 0 | 0 | 0 |
| --LYMPHOID HYPERPLASIA | | 0 | 0 | 0 | 0 | 0 | 1 |
| CAECUM | NUMBER EXAMINED: | 2 | 1 | 0 | 3 | 2 | 1 |
| --CONGESTION/HAEMORRHAGE | | 2 | 1 | 0 | 3 | 2 | 1 |
| SPLEEN | NUMBER EXAMINED: | 2 | 0 | 0 | 1 | 1 | 1 |
| --CONGESTION | | 2 | 0 | 0 | 0 | 1 | 1 |
| --ADHESION | | 0 | 0 | 0 | 1 | 0 | 0 |
| ILEO COLON | NUMBER EXAMINED: | 1 | 1 | 0 | 0 | 1 | 0 |
| --CONGESTION | | 1 | 1 | 0 | 0 | 1 | 0 |
| STOMACH | NUMBER EXAMINED: | 0 | 0 | 0 | 1 | 1 | 2 |
| --LYMPHOID HYPERPLASIA | | 0 | 0 | 0 | 0 | 1 | 1 |
| --CONGESTION/HAEMORRHAGE | | 0 | 0 | 0 | 0 | 0 | 1 |
| OVARY | NUMBER EXAMINED: | 0 | 0 | 0 | 2 | 0 | 1 |
| --OESTROUS STAGE | | 0 | 0 | 0 | 1 | 0 | 1 |
| UTERUS | NUMBER EXAMINED: | 0 | 0 | 0 | 1 | 0 | 0 |
| --OESTROUS STAGE | | 0 | 0 | 0 | 1 | 0 | 0 |
| COLON | NUMBER EXAMINED: | 0 | 0 | 0 | 2 | 1 | 0 |
| --CONGESTION/HAEMORRHAGE | | 0 | 0 | 0 | 2 | 1 | 0 |
| ILEUM | NUMBER EXAMINED: | 0 | 0 | 0 | 1 | 0 | 0 |
| --HAEMORRHAGE/CONGESTION | | 0 | 0 | 0 | 1 | 0 | 0 |
| SKIN SUBCUTIS | NUMBER EXAMINED: | 0 | 0 | 0 | 0 | 1 | 2 |
| --DERMATITIS | | 0 | 0 | 0 | 0 | 1 | 2 |
| --FOLLICULITIS | | 0 | 0 | 0 | 0 | 1 | 0 |
| DUODENUM | NUMBER EXAMINED: | 0 | 0 | 0 | 0 | 0 | 1 |
| --HAEMORRHAGE/CONGESTION | | 0 | 0 | 0 | 0 | 0 | 1 |
| ** END OF LIST ** | | | | | | | |

Individual pharmacokinetic parameters for [redacted] following a single intranasal dose [redacted] on Day 1 at a dose level of 120mg base/dose (Study D12787)

| PK Parameter | Animal Number 2315M | 2316M | 2317M | 2318M | 2327F | 2328F | 2329F | 2330F | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| $AUC_\infty$ | 3470 | 6250 | 7130 | 7160 | 9610 | 7370 | 9470 | 6770 | 7150 | 1930 |
| $AUC_t$ | 3140 | 6160 | 6940 | 7090 | 9380 | 7230 | 8430 | 6700 | 6880 | 1830 |
| $AUC_{(0-4h)}$ | 2320 | 3820 | 4890 | 4250 | 7590 | 5370 | 6360 | 4810 | 4930 | 1600 |
| $C_{max}$ | 1040 | 2320 | 2150 | 1980 | 4400 | 2740 | 4480 | 2920 | 2750 | 1180 |
| $C_{min}$ (24h,Day 1) | <5.0 | 14.8 | 21.4 | 12.3 | <5.0 | 17.9 | 56.1 | 11.4 | 13.6* | - |
| $T_{max}$ | 0.5 | 0.5 | 0.5 | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.56 | 0.18 |

Individual pharmacokinetic parameters for [redacted] following a single intranasal dose [redacted] on Day 1 at a dose level of 240mg base/dose (Study D12787)

| PK Parameter | Animal Number 2319M | 2320M | 2321M | 2322M | 2331F | 2332F | 2333F | 2334F | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| $AUC_\infty$ | 10000 | 13200 | 11800 | 16800 | 15300 | 15000 | 14800 | 18900 | 14500 | 2800 |
| $AUC_t$ | 10000 | 13000 | 11800 | 16600 | 15200 | 14900 | 14400 | 18600 | 14300 | 2710 |
| $AUC_{(0-4h)}$ | 7660 | 5520 | 10200 | 10700 | 8880 | 9510 | 9620 | 11500 | 9200 | 1880 |
| $C_{max}$ | 3470 | 1930 | 4900 | 5940 | 3710 | 3990 | 4560 | 5860 | 4300 | 1330 |
| $C_{min}$ (24h, Day 1) | 7.3 | 26.8 | 8.9 | 27.8 | 17.1 | 15.8 | 50.9 | 34.3 | 23.6 | 14.5 |
| $T_{max}$ | 1.0 | 1.0 | 1.0 | 0.5 | 0.5 | 1.0 | 1.0 | 0.5 | 0.81 | 0.26 |

PK Parameter units:

$AUC_t$, $AUC_\infty$ and $AUC_{(0-4h)}$ - ng.h/mL

$T_{max}$ - h

$C_{max}$, $C_{min}$ - ng/mL

* Median value, range <5.0 to 56.1

Individual pharmacokinetic parameters for following repeat intranasal dosing on Day 35 at a total dose level of 240mg base/dog (Study D12787)

| PK Parameter | Animal Number 2315M | 2316M | 2317M | 2318M | 2327F | 2328F | 2329F | 2330F | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| $AUC_{(0-4h)}$ (1st dose) | 1510 | 8510 | 3880 | 4460 | 4210 | 2640 | 4160 | 1440 | 3850 | 2240 |
| Total $AUC_t$ (both doses) | 8790 | 17000 | 9100 | 10200 | 9520 | 5930 | 11500 | 3820 | 9480 | 3900 |
| $C_{max}$ (1st dose) | 470 | 3210 | 1920 | 2350 | 2380 | 1410 | 2200 | 587 | 1820 | 940 |
| Total $C_{max}$ (both doses) | 2340 | 3210 | 1920 | 2350 | 2380 | 1410 | 2200 | 644 | 2060 | 761 |
| $C_{min}$ (predose, Day 35) | 23.8 | 59.3 | 74.2 | 46.4 | 55.0 | 85.8 | 25.3 | 31.0 | 50.1 | 22.8 |
| $T_{max}$ (1st dose) | 1.0 | 2.0 | 0.5 | 1.0 | 1.0 | 0.5 | 0.5 | 0.5 | 0.88 | 0.52 |
| Total $T_{max}$ (both doses) | 5.0 | 2.0 | 0.5 | 1.0 | 1.0 | 0.5 | 0.5 | 5.0 | 1.94 | 1.95 |

Individual pharmacokinetic parameters for following repeat intranasal dosing on Day 35 at a total dose level of 480mg base/dog (Study D12787)

| PK Parameter | Animal Number 2319M | 2320M | 2321M | 2322M | 2331F | 2332F | 2333F | 2334F | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| $AUC_{(0-4h)}$ (1st dose) | 3830 | 6850 | 10000 | 7610 | 10100 | 11100 | 5290 | 5000 | 7470 | 2700 |
| Total $AUC_t$ (both doses) | 9780 | 18800 | 19600 | 20800 | 21200 | 28000 | 8280 | 15000 | 17700 | 6450 |
| $C_{max}$ (1st dose) | 1740 | 3070 | 7050 | 4970 | 4250 | 4220 | 3930 | 1670 | 3860 | 1760 |
| Total $C_{max}$ (both doses) | 1740 | 3070 | 7050 | 4970 | 4250 | 4220 | 3930 | 3270 | 4060 | 1550 |
| $C_{min}$ (predose, Day 35) | 95.3 | 111 | 74.9 | 53.2 | 135 | 195 | 75.4 | 43.2 | 97.9 | 49.3 |
| $T_{max}$ (1st dose) | 1.0 | 0.5 | 1.0 | 1.0 | 1.0 | 2.0 | 0.5 | 1.0 | 1.0 | 0.46 |
| Total $T_{max}$ (both doses) | 1.0 | 0.5 | 1.0 | 1.0 | 1.0 | 2.0 | 0.5 | 5.0 | 1.50 | 1.49 |

PK Parameter units:

$AUC_t$, $AUC_\infty$ and $AUC_{(0-4h)}$ - ng.h/mL

$T_{max}$ - h

$C_{max}$, $C_{min}$ - ng/mL

**14 Day Intranasal Irritancy to Cynomolgus Monkeys**

Testing Facility:

Dates of Dosing: 9/23/86 - 10/8/86

Test Material: Note that this formulation is different from the clinical formulation. The formulation used in this study is unbuffered, whereas the clinical formulation is buffered. Test substance was supplied as two different strength solutions of (hemi-sulphate), prepared *in situ* in water (200 mg(base)/ml and 400 mg(base)/ml). These solutions were supplied in amber glass bottles. The supply was stored in the dark under refrigeration. The batches of used were: - 200 mg/ml - batch # 400 mg/ml - batch # The control was sterile water for injection, batch # 82J851. It is not clear if the vehicle solution was adjusted to take into account the sulfuric acid and sodium hydroxide required to make th *in situ*.

Stability: Historical data was supplied for formulations after storage for 5 weeks at 4 °C or 30 °C, rather than the actual conditions. Under these conditions there was a decrease in parent compound level of less than 4% for both strengths. According to the sponsor this difference is thought to be due to the sampling technique. Under these conditions there was also a ± 0.2 unit shift in pH.

Concentration Check: According to the sponsor, day 14 samples were returned to the sponsor (10/10/86) from the contract laboratory and concentration analysis carried out by reverse phase HPLC. The data was supplied as a comparison of the actual concentrations to the nominal concentration (vol 15, pg 166). The data presented is for nominal concentrations of 5, 10, 20, 40, 80 and 100 mg (base)/ml; however, the test formulations were 200 and 400 mg(base)/ml.

Animal: Wild caught cynomolgus monkeys (Macaca fascicularis) from When assigned to the study the body weights ranged from 2.20 - 2.96 kg and age was not estimated. Each animal was given 200 g of primate diet daily and, on weekdays, this was supplemented with weighed quantities of wholemeal bread and fresh fruit. Black currant juice and vitamin supplements were provided. According to the sponsor "The monkey was selected as the test model as the anatomy of the nasal structures in monkeys is more like that of man than other laboratory animal species."

Route: Intranasal dosing using a Valois metering atomising pump. According to the sponsor, drug administration occurred as follows: the animals were restrained in a supine position with its head tilted backward. The nasal adapter of the dosing device was positioned just within the external nares. The air trigger releases the spray into the nostril, the device is repositioned and the other nostril is dosed. The monkey was held in position for approximately 30 seconds after the last dose and then returned to its cage.

According to the sponsor, nasal adapters were replaced daily and new pump units were attached to new drug bottles each day; however, according to the protocol, each day fresh solutions of test substance were added to the residue in the nasal spray bottle from the previous day's dosing. The vehicle was discarded before the next dosing. The sponsor states that the nasal spray packs were stored in the dark, under refrigeration, between dosing periods each day, and removed from refrigeration and allowed to warm to room temperature shortly before dosing. The sponsor also states that one dosing unit was used to administer the vehicle control and a second unit was used to administer both concentrations of the solutions (200 mg/ml and 400 mg/ml).

Dosing Rationale: The rationale for the choice of doses or the dosing regimen was not mentioned.

| DOSING REGIMEN | | | | | | |
|---|---|---|---|---|---|---|
| group | treatment | dose mg(base) | concentration mg(base)/ml | # sprays | volume | #/sex |
| control | vehicle water for injection | 0 | 0 | 1 spray/nostril/session<br>total=2 sprays/session<br>x 4 sessions/day<br>4 sprays/nostril/day<br>total = 8 sprays/day | 0.1 ml/spray<br>total=0.2 ml/session<br>total = 0.8 ml/day | 2 |
| low dose | | 40/session<br>x 4 sessions/day<br>total = 160/day | 200 | 1 sprays/nostril/session<br>total=2 sprays/session<br>x 4 sessions/day<br>4 sprays/nostril/day<br>total = 8 sprays/day | 0.1 ml/spray<br>total=0.2 ml/session<br>total = 0.8 ml/day | 2 |
| high dose | | 80/session<br>x 4 sessions/day<br>total = 320/day | 400 | 1 spray/nostril/session<br>total=2 sprays/session<br>x 4 sessions/day<br>4 sprays/nostril/day<br>total = 8 sprays/day | 0.1 ml/spray<br>total=0.2 ml/session<br>total = 0.8 ml/day | 2 |

Total daily dosage was divided over four daily sessions, separated by 2 hours for 13 consecutive days. On day 14 all control animals and one male and one female from the low and high dose treatment groups were dosed once only (one spray to each nostril once only). The predetermined (based on consistent clinical signs) remaining one male and one female in the low and high dose treatment groups received repeated intravenous injections of the intranasal solutions diluted with normal saline in order to determine at what dose salivation would be induced. Injections of 0.5 ml were made over 30 seconds into the posterior tibial vein.

| PROTOCOL | | |
|---|---|---|
| parameter | animals tested | description |
| drug administration | all | 13 consecutive days, four dosing sessions per day, each separated by two hours. On day 14 all control animals and one male and one female from each of the two treatment groups were dosed intranasally once. The remaining one male and one female per treatment group (not randomly selected, but selected based on consistent clinical signs) received repeated intravenous injections of the intranasal solutions diluted with normal saline in order to determine the dose at which salivation would be induced. Injections of 0.5 ml were made over 30 seconds into the posterior tibial vein. |
| clinical signs | all | checked regularly throughout the working day, this included, at a minimum, observation during and immediately following dosing, and on weekdays, at 5:00 pm. |
| body weights | all | weighed weekly for the two weeks prior to treatment as well as during treatment and on day of sacrifice. |
| food consumption | all | estimated daily for one week prior to the initiation of treatment and during the dosing period. data was recorded based on weekly estimates. |
| plasma drug concentrations | - | from all animals dosed intranasally on day 14 (i.e., all control and one male and one female from each of the two treated groups) after a single dose (one spray to each nostril) at 0.25, 0.5, 1, 2, 6 and 24 hours post dose. By HPLC with electrochemical detection. |
| necropsy | all | on day 15 (males and females on separate days) all animals were anesthetized with intravenous pentobarbitone and exsanguinated. |
| histopathology | all | full compliment of tissues were immersion fixed (10% neutral buffered formalin; eye-Davidson's solution). The lungs were infused with fixative prior to immersion. Histopathology was carried out on the following tissues (5 micron, H&E): nasal passages (3 levels - anterior, middle and posterior) pharynx, larynx (4 levels), heart (including coronary arteries), trachea (3 levels - anterior, middle and posterior), carina, lungs and bronchi (all lobes). |

Results

Delivery System: The following range of volumes were delivered per spray for the control, low and high dose groups respectively: 0.18-0.19 ml, 0.19-0.20 ml and 0.17-0.19 ml.

Mortality: None

Clinical Signs: There were no recorded clinical signs in the control group. Treatment related clinical signs included: salivation, vomiting, retching, glazed appearance of eyes, nasal discharge and wiping mouth or rubbing mouth and chin on cage. Salivation occurred in each treated animal at least once per day. Incidence and severity increased with increasing dose. According to the sponsor the duration was noted to be 10-20 minutes and the onset was noted to be shorter for the higher dose. Vomiting and retching occurred in a dose related way during the first three days of dosing. There were only two incidences of retching outside this time frame in high dose treated animals. The sponsor points out that the vomitus was mostly saliva. There were dose related incidences of nasal discharge. Occasionally in the low dose group it was noted to be blood stained, which the sponsor attribute to damage due to the mechanical device. Note that no damage or irritation was noted at necropsy and the same type of device was used for the control and both treatment groups. As a clinical sign, nasal discharge is difficult to interpret. It could possible be some of the drug substance or treatment induced rhinorrhea . Recall that this sign was not present in the placebo-treated group. Although no data was provided the sponsor states that wiping of the mouth or rubbing mouth and chin on cage was seen on occasion in high dose animals and a glazed appearance in the eyes of two high dose animals were noted on treatment day 1.

Recall that one low dose treated male, one low dose treated female, one high dose treated male and one high dose treated female were given ascending intravenous doses of on day 14 of this study (rather than the intranasal doses) to see if intravenous administration could produce salivation. Salivation was observed in three of the four monkeys after the initial intravenous dose of 1 mg/kg. It is not clear whether salivation was ever seen in the remaining animal given intravenous doses up to 24 mg/kg. This high dose did result in altered behavior.

Body Weight: There was an apparent treatment related decrease in body weight gain, which appeared to be more severe in the low dose treated group, where all four animals showed a net decrease in body weight. In the high dose group two of the four animals had a net weight loss and the remaining two had a decrease in body weight gain. These results may not be easy to interpret due to the small sample size, short treatment period and the normal variability in monkey weight patterns.

Food Consumption: There appears to be a treatment related, though not strictly dose related, decrease in food consumption. Again, these results may not be easy to interpret due to the small sample size, short treatment period and the normal variability in monkey eating habits.

Macroscopic Examination: A copy of the sponsor-provided summary table follows. According to the sponsor no lesions present were attributable to All were considered incidental. Examination of the data reveals that a high dose female was noted with patchy black discoloration of the tongue, which was not examined histopathologically. Note that gross findings not related to the respiratory system or heart were not further examined.

Histopathology: A copy of the sponsor-provided summary table follows. According to the sponsor no findings were attributable tc The sponsor specifically states: "There were, in particular, no histopathological changes in the nasal passages." The sponsor notes that the finding of black pigmented macrophages in all animals, including the controls , is not uncommon in cynomolgus monkeys and, is possibly related to lung mites. No lung mites were seen on histopathology.

Examination of the data reveals that one high dose treated female was noted with focal pulmonary fibrosis and left pleural adhesion and a foci of lymphocytic infiltration into the endocardium. A low dose treated female was noted with foci of alveolar macrophages. Recall that no histopathology was carried out on gross lesions not associated with the respiratory system or the heart.

Plasma Drug Concentration: Copies of the sponsor-provided summary table and figure follow. Recall that plasma drug concentrations were determined on only one male and one female in the treatment groups. No data was supplied for the controls, only the following statement was supplied: "Analysis of a sample of control plasma taken from the control group gave apparent concentrations of base of approximately 2 ng/ml or less, attributable to a minor endogenous component." According to the sponsor: "There is some suggestion from the data that an early high plasma concentration may have been achieved prior to taking of the first sample. The subsequent peak plasma levels may be attributed to oral absorption following later ingestion of the drug solution with the time to peak between 2 and 3 hours."

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

## Macroscopic pathology incidence summary

| Group: | 1 | 2 | 3 |
|---|---|---|---|
| Compound: | Water | (200 mg/ml) | (400 mg/ml) |
| Dose (mg/day): | 0 | 160 | 320 |

| Macroscopic observations | Groups | | | | | |
|---|---|---|---|---|---|---|
| | 1♂ | 2♂ | 3♂ | 1♀ | 2♀ | 3♀ |
| | Control | Low | High | Control | Low | High |
| Lungs | | | | | | |
| - pleural adhesions | 1 | - | - | - | - | 1 |
| Liver | | | | | | |
| - pale raised areas | - | - | - | 1 | - | - |
| - pale area | - | - | - | - | - | 1 |
| - cyst containing parasite | - | - | - | 1 | - | - |
| Stomach | | | | | | |
| - depression | - | - | - | - | - | 1 |
| Caecum | | | | | | |
| - dark nodules | 2 | 2 | - | - | 1 | - |
| Colon | | | | | | |
| - dark nodule(s) | 2 | - | - | - | 1 | - |
| Skin | | | | | | |
| - adherent to muscle | 1 | - | - | - | - | - |
| - accessory nipples | - | - | - | 1 | - | - |
| Bone | | | | | | |
| - vertebra malaligned | 1 | - | - | - | - | - |
| - articular cartilage of left and right humerus, eroded | - | - | - | - | 1 | - |
| Tongue | | | | | | |
| - black | - | - | 1 | - | - | - |
| Adipose tissue | | | | | | |
| - splenuli | 1 | - | - | - | - | - |
| - cysts containing parasites | - | - | - | 1 | - | - |
| No. of monkeys examined | 2 | 2 | 2 | 2 | 2 | 2 |

## Microscopic pathology incidence summary

| Group: | 1 | 2 | 3 |
|---|---|---|---|
| Compound: | Water | (200 mg/ml) | (400 mg/ml) |
| Dose (mg/day): | 0 | 160 | 320 |

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| Monkeys on study: | 2 | 2 | 2 | 2 | 2 | 2 |
| Monkeys Logged: | 2 | 2 | 2 | 2 | 2 | 2 |
| Trachea | 2 | 2 | 2 | 2 | 2 | 2 |
| Not Remarkable | 2 | 2 | 2 | 2 | 2 | 2 |
| Lungs | 2 | 2 | 2 | 2 | 2 | 2 |
| Not Remarkable | 0 | 0 | 0 | 0 | 0 | 0 |
| Foci of black pigmented macrophages | 2 | 2 | 2 | 2 | 2 | 2 |
| Foci of alveolar macrophages | 0 | 0 | 0 | 0 | 1 | 0 |
| Focal pleural fibrosis | 0 | 0 | 0 | 0 | 0 | 1 |
| Larynx | 2 | 2 | 2 | 2 | 2 | 2 |
| Not Remarkable | 2 | 2 | 2 | 2 | 2 | 2 |
| Turbinates | 2 | 2 | 2 | 2 | 2 | 2 |
| Not Remarkable | 2 | 2 | 2 | 2 | 2 | 2 |
| Carina | 2 | 2 | 2 | 2 | 2 | 2 |
| Not Remarkable | 2 | 2 | 2 | 2 | 2 | 2 |
| Pharynx | 2 | 2 | 2 | 2 | 2 | 2 |
| Not Remarkable | 2 | 2 | 2 | 2 | 2 | 2 |
| Heart | 2 | 2 | 2 | 2 | 2 | 2 |
| Not Remarkable | 2 | 2 | 2 | 1 | 2 | 1 |
| Focus of lymphocytic infiltration | 0 | 0 | 0 | 1 | 0 | 0 |
| Foci of lymphocytic infiltration into endocardium | 0 | 0 | 0 | 0 | 0 | 1 |

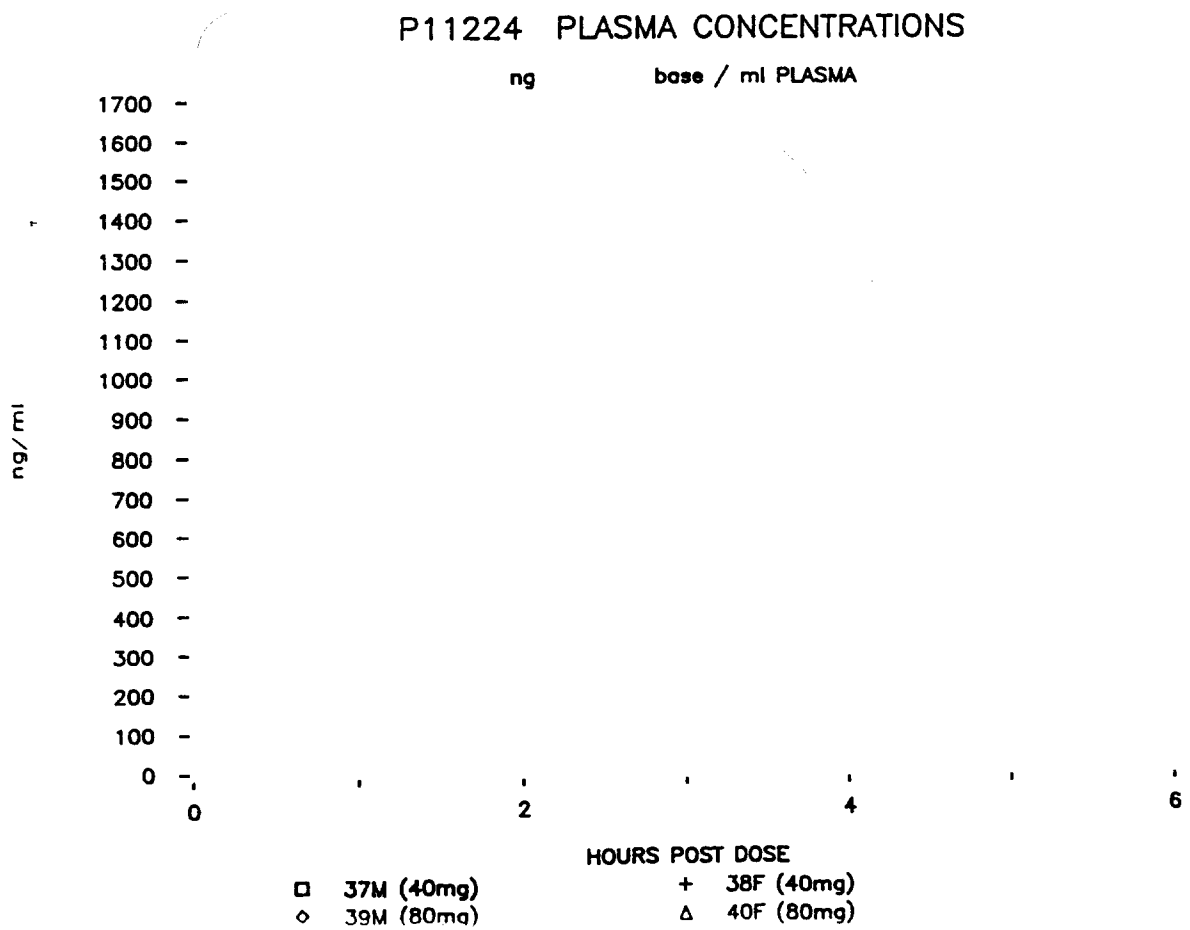
P11224 - Concentration of G base in monkey plasma (ng/ml)

Dose - 40mg intranasal administration

| Time | Animal No. 37 (M) | Animal No 38 (F) | Mean |
|---|---|---|---|
| 0.25 hour | 101 | 91 | 96 |
| 0.5 hour | 58 | 174 | 116 |
| 1 hour | 312 | 523 | 418 |
| 2 hour | 464 | 640 | 552 |
| 6 hour | 62 | 60 | 61 |
| 24 hour | 16 | 6.8 | 11 |

Dose - 80mg intranasal administration

| Time | Animal No. 39 (M) | Animal No 40 (F) | Mean |
|---|---|---|---|
| 0.25 hour | 633 | 195 | 414 |
| 0.5 hour | 335 | 294 | 315 |
| 1 hour | 608 | 1219 | 914 |
| 2 hour | 710 | 1698 | 1204 |
| 6 hour | 207 | 146 | 177 |
| 24 hour | 23 | 18 (estimated) | 21 |



**with Saccharin: 13 Week Intranasal Irritancy Study in the Beagle Dog**

Testing Facility:

Dates of Dosing: 2/25/92 -

Test Material: The test formulation used in this study is not the same as the proposed clinical formulation. The test formulation used in this study contains 2% saccharin and is unbuffered. The clinical formulation was buffered and unsweetened.

The test material was supplied by the sponsor as _..... in 2% w/v saccharin sodium BP with an expiry date of 7/31/92. When not in use, the test substances were to be stored at 17-26 °C, protected from light. The vehicle control was saccharin sodium 2% w/v - batch # F91/251A. solutions were from the follow batches: 200 mg(base)/ml - batch # F91/243A and 400 mg(base)/ml - batch # F91/244A.

Stability: Solutions remaining at the end of the study were returned to the sponsor for analysis. According to the stability data the pH decreased by a unit over the course of the study (5.1 → 4.0). For both concentrations of the test formulation, the solutions were within 4% of the nominal concentration after completion of the study.

Animal: This study employed pure bred beagles from At the initiation of treatment the males weighed 6.90 to 10.0 kg and females 6.05 to 7.60 kg and were between six and eight months of age. Animals were housed singly during the day and in pairs (by sex and group) at nights after removal of food. Animals were given 400 g of lab diet daily (any remainder removed at night) and had free access to water. Animals were assigned to treatment groups using a randomization procedure based on stratified weight. Litter mates were distributed across groups whenever possible.

Route: This study was carried out by the intranasal route using a metered pump dosing device. The animals were familiarized with the dosing procedure by sham dosing for four days prior to the initiation of treatment. According to the sponsor the dosing began with the left nostril during inspiration, with the external nares elevated. All animals had their eyes covered during dosing. One dosing pump was allocated to each dose group daily. One vial contained sufficient volume to dose an entire group. Data were collected to determine the delivery per actuation of the pump device from five devices from each treatment group. The weight (mg) of the delivered formulation per actuation varied between 94 to 113 mg, compared to the nominal 100 mg.

Dosing Rationale: According to the sponsor the choice of doses was based on a previous study (# D12278) which demonstrated that the maximum "practicable" intranasal dose of to be 240 mg(base)/dog, b.i.d.

At the time this current study was being conducted, the sponsor was pursuing a clinical formulation in 2% w/v saccharin. The volume of each spray was the same as that proposed for use in humans and the number of doses per nostril was chosen to "provide a suitable average above a likely therapeutic dose."

| DOSING REGIMEN | | | | | | |
|---|---|---|---|---|---|---|
| group | treatment | dose (mg(base) | concentration (mg(base)/ml) | # sprays | volume | #/sex |
| control | saccharin sodium 2% w/v | 0 per session<br>X 2 sessions total = 0/day | 0 | 3/nostril/session X 2 daily sessions | 0.1 ml/spray | 4 |
| low dose | in saccharin sodium 2% w/v | 120 per session<br>X 2 sessions/day total = 240/day | 200 | 3/nostril/session X 2 daily sessions | 0.1 ml/spray | 4 |
| high dose | in saccharin sodium 2% w/v | 240 per session<br>X 2 sessions/day total = 480/day | 400 | 3/nostril/session X 2 daily sessions | 0.1 ml/spray | 4 |
| There were two dosing sessions daily, separated by 4-6 hours, except on day 1 on which only one dose was administered to allow for PK studies. | | | | | | |

| PROTOCOL | | |
|---|---|---|
| parameter | animals tested | description |
| drug administration | all | Once per day on day 1 and twice per day for 90-91 consecutive days, excluding the day of necropsy. Daily dosing sessions were separated by 4-6 hours. |
| clinical signs | all | In the morning prior to feeding and again in afternoon. On day one they were observed to establish the range and duration of signs, thereafter they were observed throughout the day. |
| body weight | all | Recorded weekly throughout and prior to necropsy |
| food consumption | all | Estimated and recorded daily, weekly consumption calculated. |
| plasma drug concentration | control<br>low & high | From jugular vein<br>day 1, day 35 & week 13 - five minutes after the first dose.<br>day 1 - predose, 5 and 30 min post, 1, 2, 4, 6, 8 and 24 hours post<br>day 35 - pre dose, 5 and 30 min post, 1, 2, and 4 hours post first dose. The four hour sampling was followed immediately by the second dose after which blood was sampled at 5 and 30 minutes post and 1, 2, 4, 6 , 8 and 10 hours post second dose.<br>week 13 - once prior to first daily dose and one hour post second daily dose. the two daily doses were separated by the same time as on day 35. |
| necropsy | all | Animals were anesthetized with intravenous sodium thiopentone (after an overnight fast) followed by exsanguination. Nasal passages were fixed intact and not examined macroscopically. Necropsies were carried out over two days in replicate order, with a similar number of males and females per group killed on each day. |
| organ weights | all | Adrenals, brain (including brainstem), heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes and epididymides, thyroids and parathyroids. |
| histopathology | all | Basically a full compliment of tissues were immersion fixed in 10% neutral buffered formalin (except eyes in Davidson's fluid). Only the following tissues were paraffin embedded, sectioned at 5 microns, and stained with H&E: larynx with pharynx, lungs (with mainstem bronchi), lymph nodes (retropharyngeal and bronchial), nasal passages, tongue, trachea and bifurcation, all gross lesions. |

Results

Mortality: None

Clinical Signs: Treatment related clinical signs include: mydriasis, salivation, high pitched vocalization, and less frequently, vomiting, lachrymation, and ocular opacity. The sponsor provided incidence information only for treatment related mydriasis, salivation and high pitched vocalization. These three signs did not occur in the control group. Salivation occurred in both the low and high dose treated groups in a dose related fashion. According to the sponsor the onset was usually immediately post dosing, and occasionally during dosing, with a duration of 1 to 1.5 hours. Mydriasis was noted in every treated animal throughout the study. The sponsor notes that the onset for mydriasis was usually 1 to 2 hours after the first dosing of the day and was still present at the end of the work day. It was noted that mydriasis was not present prior to the next mornings dose. Periods of high pitched vocalization occurred in all treated animals intermittently throughout the study. There was no clear evidence of tolerance for mydriasis, salivation or vocalization. According to the sponsor, the onset for this sign was one to two hours after dosing, and the periods were generally of short duration. Although there was no specific data provided, the sponsor states that there were also occasional incidences of vomiting, lachrymation and a high dose female was noted with ocular opacity during the first week of treatment.

Body Weight: There was no clear effect of treatment on body weight gain.

Food Consumption: There was no clear effect of treatment on food consumption.

Gross Pathology: According to the sponsor: "There was no evidence of a gross lesion attributable to the test material administration." However, recall that there was no gross examination of the nasal cavities. The sponsor does mention the occurrence of dark or red foci of the GI tract, enlarged or cystic ovaries and vagina and isolated occurrences of adhesions in the lung (a high dose male), dark lungs and pale areas in the lung.

Organ Weights: The sponsor's analysis was based on statistically significant differences among treatment groups. According to the sponsor "There was no conclusive evidence that treatment affected the weight of organs from treated animals when compared with those from controls." The sponsor does point out that the pituitaries from high dose males were statistically heavier than controls (due to increases in two animals 2869M and 2870M). The sponsor states that this finding is equivocal since there were no histological findings and no similar effect in females.

Examination of the individual animal data revealed the following patterns. Due to the small sample size, the inherent variability of this parameter and lack of pathology data for most organs, it would be difficult to attribute the alterations to treatment.

| Changes in Organ Weights | | | | |
|---|---|---|---|---|
| | low dose male | high dose male | low dose female | high dose female |
| adrenals | ↑ absolute & relative | ↑ absolute & relative | | ↑ absolute & relative |
| kidneys | ↓ absolute & relative | ↓ absolute & relative | ↑ absolute | ↑ absolute |
| pituitary | | ↑ absolute & relative | | |
| thyroid | ↑ absolute & relative | | ↑ absolute & relative | ↑ absolute & relative |
| lung | | ↑ relative (in one) | | |
| testes & epididymides | ↑ relative | ↑ relative | | |
| spleen | ↓ absolute & relative | ↓ absolute & relative | ↑ absolute & relative | |

Histopathology: A copy of the sponsor-provided summary table follows. The sponsor suggests that the erosion in the nasal cavity in a high dose treated female and the epithelial hyperplasia in the maxilloturbinates in another high dose treated female were possibly the result of minor irritation due t According to the sponsor there was no other evidence of irritancy or toxicity attributable to the The sponsor points out that the higher incidence of fibrosing alveolitis seen in GR43175 treated females was not treatment related, since it was considered "as a resolving stage of a pre-existing inflammation."

Plasma Drug Concentration: Copies of the sponsor-supplied summary tables follow. According to the sponsor there was no sex difference so all conclusions were based on sex combined data. The sponsor looked at the plasma concentration in the control plasmas drawn 5 minutes post dose for days 1, 35 and week 13. All plasma levels in the control treated animals were below detection levels (<5 ng/ml). According to the sponsor, on several occasions during sampling on day 1 and during week 13, samples were drawn up to eight minutes later than stated. Note that in the summary table $C_{max}$ is not a calculated $C_{max}$, but simply the highest concentration sampled per animal. The $C_{max}$ and AUC increase with increasing dose but to a lesser degree than expected; however, given the variability of the data it would be difficult to make any definitive statements about this. The $C_{min}$ values increase somewhat between treatment day 1 and day 35; however, there is no further increase between day 35 and week 13. Therefore, there is no clear evidence of accumulation.

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

| ORGAN AND FINDING DESCRIPTION | | --- NUMBER-OF-ANIMALS-AFFECTED --- | | | | | |
|---|---|---|---|---|---|---|---|
| SEX: | | -----MALE---- | | | ----FEMALE--- | | |
| GROUP: | | -1- | -2- | -3- | -1- | -2- | -3- |
| NUMBER: | | 4 | 4 | 4 | 4 | 4 | 4 |
| ** TOP OF LIST ** | | | | | | | |
| LUNG | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --EXTRA SAMPLE | | 0 | 1 | 0 | 0 | 0 | 0 |
| --ECTOPIC BONE | | 0 | 0 | 0 | 0 | 1 | 0 |
| --OEDEMA | | 3 | 1 | 1 | 1 | 1 | 2 |
| --HAEMORRHAGE | | 0 | 4 | 1 | 2 | 2 | 2 |
| --MICROTHROMBUS | | 0 | 0 | 0 | 1 | 0 | 0 |
| --FOAMY HISTIOCYTES | | 0 | 1 | 0 | 1 | 0 | 0 |
| --PIGMENTED HISTIOCYTES | | 0 | 1 | 0 | 0 | 0 | 0 |
| --INFLAMMATORY CELL FOCI | | 4 | 3 | 3 | 1 | 3 | 2 |
| --PNEUMONITIS | | 4 | 3 | 4 | 3 | 4 | 4 |
| --BRONCHITIS/BRONCHIOLITIS | | 3 | 3 | 3 | 3 | 4 | 4 |
| --FIBROSING ALVEOLITIS | | 0 | 0 | 0 | 1 | 2 | 4 |
| --VASCULITIS | | 0 | 0 | 0 | 1 | 1 | 0 |
| --GRANULOMA | | 1 | 2 | 2 | 0 | 1 | 2 |
| --PLEURAL FIBROSIS/ADHESION | | 0 | 0 | 1 | 0 | 0 | 0 |
| TONGUE | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --INFLAMMATORY CELL FOCI | | 2 | 3 | 3 | 2 | 2 | 3 |
| --GRANULOMA | | 1 | 0 | 0 | 0 | 1 | 0 |
| TRACHEA | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --INFLAMMATORY CELL FOCI | | 3 | 1 | 2 | 1 | 1 | 0 |
| --FOCUS OF TRANSITIONAL EPITHELIUM | | 3 | 1 | 2 | 3 | 2 | 4 |
| LARYNX | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --INFLAMMATORY CELL FOCI | | 1 | 2 | 0 | 0 | 2 | 1 |
| PHARYNX | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --INFLAMMATORY CELL FOCI | | 1 | 1 | 1 | 1 | 0 | 0 |
| BRONCHIAL LN | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --PIGMENT | | 2 | 3 | 3 | 3 | 4 | 4 |
| BRONCHIAL LN | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --HAEMORRHAGE | | 0 | 0 | 1 | 1 | 1 | 0 |
| --REACTIVE | | 1 | 0 | 1 | 0 | 0 | 2 |
| PHARYNGEAL LN | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --HAEMORRHAGE | | 0 | 0 | 0 | 1 | 0 | 0 |
| NASAL CAVITY | NUMBER EXAMINED: | 4 | 4 | 4 | 4 | 4 | 4 |
| --CYSTIC GLANDS | | 0 | 0 | 0 | 0 | 1 | 0 |
| --FOCAL EROSION | | 0 | 0 | 0 | 0 | 0 | 1 |
| --EPITHELIAL HYPERPLASIA | | 0 | 0 | 0 | 0 | 0 | 1 |
| SKIN SUBCUTIS | NUMBER EXAMINED: | 0 | 0 | 0 | 0 | 1 | 0 |
| --DERMATITIS | | 0 | 0 | 0 | 0 | 1 | 0 |
| EAR | NUMBER EXAMINED: | 0 | 1 | 0 | 0 | 0 | 0 |
| --DERMATITIS | | 0 | 1 | 0 | 0 | 0 | 0 |
| SPLEEN | NUMBER EXAMINED: | 1 | 0 | 0 | 0 | 0 | 0 |
| --HAEMOSIDEROTIC NODULES | | 1 | 0 | 0 | 0 | 0 | 0 |
| CAECUM | NUMBER EXAMINED: | 0 | 3 | 0 | 2 | 0 | 1 |
| --CONGESTION/HAEMORRHAGE | | 0 | 3 | 0 | 1 | 0 | 0 |
| --CAECITIS | | 0 | 1 | 0 | 0 | 0 | 0 |
| KIDNEY | NUMBER EXAMINED: | 0 | 0 | 1 | 0 | 0 | 0 |
| --MINERALISATION | | 0 | 0 | 1 | 0 | 0 | 0 |
| OVARY | NUMBER EXAMINED: | 0 | 0 | 0 | 2 | 1 | 1 |
| --CORPORA LUTEA | | 0 | 0 | 0 | 2 | 1 | 1 |
| ** END OF LIST ** | | | | | | | |

**Individual pharmacokinetic (PK) parameters for following a single intranasal administration of on Day 1 at a dose-level of 120mg base/dog. Protocol D13342**

| PK Parameter | 2865M | 2866M | 2867M | 2868M | 2877F | 2878F | 2879F | 2880F | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| $AUC_{\infty}$ | 6710 | 6550 | 5250 | 7480 | 9110 | 7700 | 8560 | 7350 | 7340 | 1200 |
| $AUC_t$ | 6390 | 6440 | 5120 | 7390 | 8970 | 7660 | 8500 | 7270 | 7220 | 1230 |
| $AUC_{(0-4h)}$ | 3180 | 2790 | 2810 | 4640 | 4760 | 4730 | 6770 | 4770 | 4310 | 1340 |
| $C_{max}$ | 1380 | 912 | 1490 | 1570 | 1980 | 1620 | 4880 | 1920 | 1970 | 1220 |
| $C_{min}$ (24h sample) | 33.4 | 19.4 | 17.4 | 14.4 | 21.8 | 7.7 | 9.0 | 11.4 | 16.8 | 8.3 |
| $T_{max}$ | 1.0 | 2.0 | 0.5 | 2.0 | 0.5 | 1.0 | 0.5 | 0.5 | 1.0 | 0.65 |

**Individual pharmacokinetic (PK) parameters for following a single intranasal administration of l on Day 1 at a dose-level of 240mg base/dog. Protocol D13342**

| PK Parameter | 2869M | 2870M | 2871M | 2872M | 2881F | 2882F | 2883F | 2884F | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| $AUC_{\infty}$ | 17700 | 6700 | 13700 | 9330 | 19000 | 7550 | 20800 | 14100 | 13600 | 5350 |
| $AUC_t$ | 17600 | 6570 | 13600 | 8940 | 18600 | 7340 | 20600 | 14000 | 13400 | 5350 |
| $AUC_{(0-4h)}$ | 6760 | 3310 | 6940 | 5920 | 9710 | 3670 | 12700 | 9490 | 7310 | 3190 |
| $C_{max}$ | 2050 | 1870 | 4030 | 2200 | 4480 | 2160 | 6700 | 4530 | 3500 | 1720 |
| $C_{min}$ (24h sample) | 24.7 | 19.3 | 22.6 | 34.3 | 55.2 | 30.3 | 46.3 | 23.7 | 32.1 | 12.7 |
| $T_{max}$ | 1.0 | 0.5 | 0.5 | 1.0 | 1.0 | 0.5 | 1.0 | 0.5 | 0.75 | 0.27 |

PK Parameter units:

| | |
|---|---|
| $AUC_t$, $AUC_{\infty}$, $AUC_{(0-4h)}$ | ng.h/mL |
| $C_{max}$, $C_{min}$ | ng/mL |
| $T_{max}$ | hours |

**Individual pharmacokinetic (PK) parameters for following a repeat intranasal administration o √ on Day 35 at a dose-level of 120mg base/dog bid. Protocol D13342**

| PK Parameter | 2865M | 2866M | 2867M | 2868M | 2877F | 2878F | 2879F | 2880F | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| $AUC_t$ | 10300 | 7200 | 6780 | 9890 | 11400 | 14200 | 12200 | 12900 | 10600 | 2620 |
| $AUC_{(0-4h)}$ | 3370 | 3060 | 2850 | 3520 | 5490 | 6400 | 5790 | 7010 | 4690 | 1660 |
| $C_{max}$ (post 1 dose) | 1730 | 1750 | 1870 | 2480 | 2670 | 4140 | 3580 | 2720 | 2620 | 876 |
| $C_{max}$ (overall) | 2410 | 1750 | 1870 | 2480 | 2670 | 4140 | 3580 | 2720 | 2700 | 808 |
| $C_{min}$ (pre-dose, Day 35) | 61.8 | 43.2 | 28.3 | 34.5 | 67.4 | 55.4 | 26.0 | 70.7 | 48.4 | 17.8 |
| $T_{max}$ (post 1st dose) | - | 0.5 | 0.5 | 0.5 | 1.0 | 0.5 | 0.5 | 2.0 | 0.79 | 0.57 |
| $T_{max}$ (post 2nd dose) | 1.0 | - | - | - | - | - | - | - | - | - |

**Individual pharmacokinetic (PK) parameters for following a repeat intranasal administration of on Day 35 at a dose-level of 240mg base/dog bid. Protocol D13342**

| PK Parameter | 2869M | 2870M | 2871M | 2872M | 2881F | 2882F | 2883F | 2884F | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| $AUC_t$ | 19000 | 12100 | 10200 | 17900 | 9920 | 8150 | 25500 | 14000 | 14600 | 5840 |
| $AUC_{(0-4h)}$ | 7570 | 7210 | 4210 | 7560 | 2840 | 2980 | 10400 | 6870 | 6210 | 2630 |
| $C_{max}$ (post 1 dose) | 4120 | 3880 | 2190 | 5710 | 1060 | 1280 | 5380 | 2650 | 3280 | 1770 |
| $C_{max}$ (overall) | 4120 | 3880 | 2190 | 5710 | 2190 | 1280 | 5380 | 2650 | 3430 | 1600 |
| $C_{min}$ (pre-dose sample) | 53.4 | 39.8 | 93.5 | 55.1 | 47.0 | 103 | 31.6 | 82.8 | 63.3 | 26.3 |
| $T_{max}$ (post 1st dose) | 0.5 | 0.5 | 0.5 | 0.5 | - | 0.5 | 1.0 | 1.0 | 0.64 | 0.24 |
| $T_{max}$ (post 2nd dose) | - | - | - | - | 1.0 | - | - | - | | |

PK Parameter units:

| | |
|---|---|
| $AUC_t$, $AUC_{\infty}$, $AUC_{(0-4h)}$ | ng.h/mL |
| $C_{max}$, $C_{min}$ | ng/mL |
| $T_{max}$ | hours |

## Pilot 8 Day Inhalation Study in AHA Rats

Testing Facility: unstated
This study was a non-GLP pilot study carried out to determine the dose/exposure for the more definitive 35 day study.

Dates of Dosing: unstated

Test Material: The test formulation used in this study was not the same as that proposed for marketing. The study formulation contains preservatives and is unbuffered. The proposed clinical formulation to be marketed is non-preserved and buffered. Test formulations were prepared at the testing facility. Test article concentration of 200 mg(base)/ml of was formed *in situ* from (batch # C825/136/1), sulfuric acid and sodium hydroxide in water. The preservatives benzalkonium chloride (0.2% by volume) and phenylethyl alcohol (4% by weight) were present. The vehicle control consisted of benzalkonium chloride, phenylethyl alcohol, sodium hydroxide and sulphuric acid in water for irrigation.

Stability: According to the sponsor the test article formulation should be stable at room temperature for up to 28 days (no notation of protection from light), based on historical data.

Concentration Check: According to the sponsor testing was carried out on day 1, both predose and post dose. Concentration analysis ranged from 193.1 - 196.5 mg (base)/ml compared to the nominal value of 200 mg(base)/ml.

Chamber Atmosphere Analysis: According to the sponsor the mass median aerodynamic diameter (MMAD) was 2.55 and sigma g was 1.79. The chamber concentrations varied from 680 - 1984 µg/l (mean 1347) for group 3 and 1130 - 1912 µg/l (mean 1405) for group 4.

Animals: from Glaxo Research Ltd were used. At the initiation of treatment males weighed 302.0 - 493.5g and females weighed 200.1 - 252.6g. They were housed in groups of five by sex and treatment group. For four days prior to dosing animals were acclimatized to the dosing procedure (tube). In general food and water was freely accessible.

Route: Inhalation. According to the sponsor intranasal administration to rats is not 'practicable'; therefore, inhalation was chosen as the best approximation available. Animals were dosed using a snout only exposure system employing an Acorn System22 nebulizer. Fresh solutions were used for each exposure period.

Dosing Rationale: Based on a study in cynomolgus monkeys (Study # P11224, report WPT/87/060) which demonstrated intranasal administration of 200 and 400 mg(base) GR43175 resulted in no apparent irritation of the nasal cavity and respiratory system.

| DOSING REGIMEN | | | | | | |
|---|---|---|---|---|---|---|
| group | duration of exposure (hours) | | | (base) | | #/sex sacrificed after 8-9 days |
| | | vehicle | air | aerosol (mg/(base)l) | estimated dose (mg/kg/day) | |
| 1 - air control | 0 | 0 | 2 | 0 | 0 | 5 |
| 2 - vehicle control | 0 | 2 | 0 | 0 | 0 | 5 |
| 3 - low dose | 1 | 0 | 0 | 1.35 | 45.5 - ♂ and 52.7 - ♀ | 5 |
| 4 - high dose | 2 | 0 | 0 | 1.41 | 91.5 - ♂ and 111.3 - ♀ | 5 |

| PROTOCOL | | |
|---|---|---|
| parameter | animals tested | description |
| treatment | all | once per day for 8 (male) or 9 (female) consecutive days |
| clinical signs | all | at least twice daily during the dosing period |
| body weight | all | days 1, 5, 8 and prior to necropsy |
| hematology | all | blood sampled during necropsy (exsanguination)<br>femoral marrow smears were prepared but not examined<br>hemoglobin, hematocrit, erythrocyte count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, reticulocyte count (films prepared but not examined), total leukocyte count and differential, cellular morphology, platelet count, prothrombin time, activated partial thromboplastin time. |
| clinical chemistry | all | blood sampled during necropsy (exsanguination)<br>alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, urea, creatinine, total protein, albumin, cholesterol, triglycerides, glucose, sodium, potassium, calcium, chloride, inorganic phosphate. |
| urinalysis | all | sampled day 4 (food withheld)<br>water consumption, urine volume, pH, glucose, protein, blood, urobilinogen, ketones, bilirubin, nitrite, specific gravity, leukocytes, microscopic examination of urinary sediment. |
| plasma drug levels | all | as soon as possible at the end of the exposure period on days 1 and 8<br>samples sent to Glaxo for analysis |
| necropsy | all | killed on day after final dose following exsanguination under enflurane anesthesia, all subjected to a full macroscopic examination. |
| histopathology processing | all | samples of the following organs were immersion fixed in 10% buffered formalin, embedded, sectioned, stained and examined by microscopy: trachea, liver, larynx, lungs, kidneys, head (for nasal chambers), heart, femur (& bone marrow), mediastinal lymph node |
| histopathology examination | gr 2-4 | all sampled tissues from study animals given either drug or vehicle control. |

Results

Mortality: One vehicle control female died on day 1 in the restraining tube during blood collection with no identifiable cause of death.

One high dose male was sacrificed on day 4 for humane reasons. On day 2 this animal demonstrated hunched, low posture, labored breathing, gasping, pale extremities, half-closed eyes, red nasal staining and was cold to the touch. On day 3 it demonstrated labored, noisy breathing. At necropsy the animal was noted with foamy macrophages in the lung, alveolar hemorrhage, ulceration of the laryngeal epithelium, hyperplasia of the epithelial lining of the arytenoid processes of the larynx, widespread submucosal acute inflammation of the larynx, and squamous metaplasia of the ventral meatus of the nasal cavity. Clinical chemistry and hematologic evaluation carried out prior to sacrifice revealed notably decreased WBCs, neutrophils and lymphocytes, somewhat increased serum glucose and slightly decreased serum calcium values.

Clinical Signs: Red nasal staining occurred in all treatment groups, with no association to treatment. Noisy/croaky breathing occurred in a low dose treated male and a high dose treated female, in addition to the high dose treated male sacrificed on day 4. Facial fur was occasionally stained in the high dose treated groups. The sponsor also noted occasional tense behavior and unkempt coat in treated animals.

Body Weight: Most animals in the study demonstrated a weight loss between days 1 and 9/10 with no clear related differences.

Hematology: Since this study is a small non-GLP pilot study, this parameter will not be analyzed here in depth. A quick review of the data reveals a decrease in WBCs and lymphocytes for vehicle control and low and high dose treated males (most notable in the low and high dose treated males) and the low and high dose treated females when compared to air control. Neutrophils were decreased in low dose males and low and high dose females and monocytes were decreased in vehicle control, low and high dose treated males and low dose treated females. There was also a decrease in platelet values in some low and high dose treated males, which was not present in females. Prothrombin time increased in high dose treated males and low and high dose treated females. There was not clear pattern for changes in activated partial thromboplastin time.

Basically the sponsor analyzes the data on the basis of statistically significant group differences. The sponsor points out several parameters for which the difference between treated groups and the air control attained statistical significance; however, the difference between the treated groups and vehicle control did not attain significance and, therefore, the changes cannot be attributed to treatment. The sponsor does note that mean prothrombin time is increased in the high dose males when compared to both the air and vehicle control groups.

Clinical Chemistry: Again, since this study is a small non-GLP pilot study, this parameter will not be analyzed here in depth. A quick review of the data reveals isolated decreases in potassium and inorganic phosphate values in low and mid dose treated females.

The sponsor analyzes the data on the basis of statistically significant group differences. The sponsor points out several parameters for which the difference between treated groups and the air control attained statistical significance; however, the difference between the treated groups and vehicle control did not attain significance and, therefore, the changes cannot be attributed to treatment.

Urinalysis: There was a suggestion of a treatment related increase in specific gravity in the treated animals, especially in females, which was not accompanied by a significant change in water consumption or urine output. There was also a suggestion of increased urinary ketones in low and high dose treated females.

Post Mortem: The sponsor did not provide a summary histopathology table as part of this non-GLP study. A copy of the reviewer generated table follows. Recall that the sponsor did not conduct a histopathological exam of tissues from the air control group. Therefore, all comparisons are based on animals exposed to the preservative combination. Of note is the extent of apparent treatment related damage in the high dose treated male sacrificed after 3 days of drug exposure (alveolar hemorrhage, foamy macrophage aggregate in the lung, widespread submucosal acute inflammation of the larynx, epithelial hyperplasia and ulceration of the larynx, and squamous metaplasia of the lining of the ventral meatus of the nasal cavity. These signs, plus epithelial hyperplasia on the arytenoid process and squamous metaplasia of the larynx, represent the treatment related pathology.

The sponsor states that these alterations were signs of irritancy in the upper respiratory tract. The sponsor did note the unusual occurrence of necrosis of the caudal lobe of the liver in two rats (a vehicle control female and a low dose female), and stated that it was suggestive of infarction, noted possible causes included vascular obstruction and torsion of the lobe.

Plasma Drug Levels: Systemic exposure to ... was achieved, although there was considerable variation among animals. The sponsor notes that plasma drug concentrations increased with increasing exposure time on day 8 but not on day 1.

| Plasma Levels | | | | | |
|---|---|---|---|---|---|
| group | sex | day 1 | | day 8 | |
| | | range | mean ± SD | range | mean ± SD |
| low dose | male | | 2.4 ± 1.6 | | 1.4 ± 0.51 |
| | female | | 1.6 ± 0.68 | | 1.9 ± 0.45 |
| | combined | | 2.0 ± 1.2 | | 1.7 ± 0.52 |
| high dose | male | | 1.6 ± 0.42 | | 2.7 ± 0.4 |
| | female | | 3.2 ± 1.2 | | 5.9 ± 2.1 |
| | combined | | 2.3 ± 1.2 | | 4.5 ± 2.3 |
| HPLC with UV detection | | | | | |

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| HISTOPATHOLOGY RESULTS FROM PRELIMINARY STUDY IN RATS | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | males | | | females | | |
| | | vehicle | low dose | high dose | vehicle | low dose | high dose |
| lung | alveolar mononuclear cell infiltrate | 2/5 | | | | 1/5 | 1/5 |
| lung | perivascular mononuclear cell infiltrate | 2/5 | 3/5 | 2/5 | 2/5 | 1/5 | 3/5 |
| lung | foamy macrophage aggregate | | 1/5 | 2/5 (*) | | | |
| lung | alveolar hemorrhage | | | 1/5 (*) | | | |
| lung | hemosiderosis | | | 1/5 | | | |
| larynx | submucosal inflammatory cell infiltrate | 1/5 | | 1/4 | | | 2/5 |
| larynx | widespread submucosal acute inflammation | | | 1/4 (*) | | | |
| larynx | epithelial hyperplasia on arytenoid process | | 5/5 | 1/4 | | 4/5 | 5/5 |
| larynx | epithelial hyperplasia (ventral region) | | 1/5 | 3/4 (*) | | | 5/5 |
| larynx | squamous metaplasia | | 1/5 | 1/4 | | 1/5 | 5/5 |
| larynx | mononuclear cell focus in ventral pouch | | | 1/4 | | | |
| larynx | ulceration in ventral region | | | 1/4 (*) | | | |
| larynx | small glandular cyst(s) ventral pouch | | 2/5 | | | | |
| trachea | submucosal lymphocyte focus at bifurcation | | 1/5 | | | | |
| nasal cavity | squamous metaplasia lining of the ventral meatus | | | 1/5 (*) | | | |
| liver | mononuclear cell infiltrate | 2/5 | 3/5 | 1/5 | 2/5 (#) | | 1/5 |
| liver | massive hemorrhagic necrosis of caudate lobe | | | | 1/5 | 1/5 | |
| liver | slight bile duct proliferation | | | | 1/5 | | |
| liver | capsular fibroplasia | | | | 1/5 | 1/5 | |
| liver | fibroplasia in surviving portal tracts | | | | | 1/5 | |
| liver | focal capsular thickening | | | | | 1/5 | |
| kidney | hydronephrosis (unilateral or bilateral) | | 1/5 (u) | 3/5 (u) (*) | 1/5 (b) | | 1/5 (b) |
| kidneys | nephrocalcinosis | | | | 1/5 | | |
| kidneys | medullary tubular cast | | 1/5 | | | | |
| kidneys | focal cortical tubular dilatation | | | 1/5 | | | |
| kidneys | cortical tubular cast | | | 1/5 | | | 1/5 |
| kidneys | interstitial inflammatory cell infiltrate | | | | | | 1/5 |
| heart | focal mononuclear cell infiltrate | | | 1/5 | | | |
| bone marrow | hemorrhage on surface | | | | 1/5 | | |
| lymph nodes | slight hemosiderosis | | | | | 1/5 | |

(*) - refers to incidence which includes the high dose male, killed on day 3 due to illness
(#) - refers to incidence which includes a control female which died on day 1

## 35 Day Inhalation Toxicity to Rats

Testing Facility: (1)
(

(3) Glaxo Group Research Ltd. (GLP with exceptions: report not audited by QA and no stability report of test article or test article carrier mixture) (plasma)

Dates of Dosing: initiation: 8/15/90 continued for 35-36 consecutive days

Test Material: The test formulation used in this study was not the same as that proposed for marketing. The study formulation contains preservatives and is unbuffered. The proposed clinical formulation to be marketed is non-preserved and buffered. Aqueous solutions c (hemi-sulfate) were formed *in situ* from (batch # C1032/195/1) and sulphuric acid in water. The test formulation containe at 200 mg(base)/ml with benzalkonium chloride (0.2% by volume) and phenylethyl alcohol (4% by weight) in sterile water for irrigation (at pH - 5.5). The vehicle contained the benzalkonium chloride, phenylethyl alcohol and sulphuric acid and sodium hydroxide solution in water. Drug solutions were made up at the testing facility as necessary (1-2 week intervals), stored protected from light and at room temperature. According to the sponsor, under these conditions the formulation would have a shelf life of 56 days.

Stability: There was no report for the stability of the test formulations under the conditions of the assay.

Concentration Check: The concentration of the test formulation was checked five times during the course of the study. Values ranged from 98% - 113% of the nominal value.

Chamber Atmosphere Analysis: For the low, mid and high dose respectively the aerosol mean concentrations (based on daily values) were: 1.30 ± 0.17, 1.32 ± 0.17 and 1.29 ± 0.16 mg(base)/l. According to the sponsor there was minimal variation in aerosol particle size distribution among the groups and therefore was probably of no physical importance. The sponsor notes that the approximate mass median aerodynamic diameter was 2.2 micromolar with a geometric standard deviation of 2.10. The sponsor states: "For the purposes of dose estimation, 94% by weight of the particles were less than 7$\mu$m and considered to be inhalable." The mean daily volumes of solution aerosolized (ml) for the vehicle control, low, mid and high dose groups were: 53.5 ± 0.95, 13.2 ± 0.62, 27.4 ± 0.68 and 53.0 ± 1.17 respectively.

Animals:

rats from Glaxo Group Research, Ware, UK were used. They were approximately five weeks of age at the initiation of treatment, having group mean body weights of 223-225g for males and 194-196 g for females. Animals were randomly assigned to treatment groups using a body weight stratification. They were housed 5/sex with no access to food or water during the inhalation procedure; however, at all other times, they had free access to both food and water.

Route: Inhalation of aerosol solution by snout exposure only. Dose was varied by varying the length of the exposure period to the test compound (15, 30 or 60 minutes) for 35 or 36 consecutive days.

Dosing Rationale: Based on the 8 day pilot study (# R12285)

| DOSING REGIMEN | | | | | | | |
|---|---|---|---|---|---|---|---|
| group | duration of exposure (minutes) | | | (base) | | #/sex sacrificed after 35 days | # /sex retained for 14 recovery |
| | | vehicle | air | aerosol @ (mg/(base)l) | estimated dose* (mg/kg/day) | | |
| 1 - air control | 0 | 0 | 60 | 0 | 0 | 10 | 5 |
| 2 - vehicle control | 0 | 60 | 0 | 0 | 0 | 10 | 5 |
| 3 - low dose | 15 | 0 | 45 | 1.30 | 11.5 | 10 | 0 |
| 4 - mid dose | 30 | 0 | 30 | 1.32 | 23.3 | 10 | 0 |
| 5 - high dose | 60 | 0 | 0 | 1.29 | 45.5 | 10 | 5 |

** estimated from the respirable fraction (94%) of the aerosol @ target concentration was 1.5 mg(base)/l

| PROTOCOL | | |
|---|---|---|
| parameter | animals | description |
| treatment | all | once per day for 35 (male) or 36 (female) consecutive days |
| clinical signs | all | during transfer to and from dosing apparatus. detailed examination weekly, especially for signs of audible respiratory noise. |
| body weight | all | one week prior to treatment then once weekly thereafter. |
| food consumption | all | by cage weekly starting one week prior to treatment |
| ophthalmoscopy | all | prior to allocation to treatment group using a Keeler indirect ophthalmoscope on dilated eyes, then once again during week 5. |
| hematology | all main study | day 14 and day 30 (after overnight food and water fast)<br>packed cell volume, hemoglobin, red blood cell count, mean cell volume, total white blood cell count and differential, cellular morphology, platelet count, throbotest. |
| clinical chemistry | all main study | day 14 and day 30 (after overnight food and water fast)<br>glucose, alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin, urea nitrogen, creatinine, alkaline phosphatase, total bilirubin, sodium, potassium, calcium, inorganic phosphorus, chloride, cholesterol |
| urinalysis | all main study | day 15 and day 31 (during an overnight food and water fast)<br>volume, pH, specific gravity, protein, glucose, ketones, bile pigments, urobilinogen, heme pigments, microscopy of sediment. |
| plasma drug levels | 5/sex gr 2-5 | as soon as possible at the end of the exposure period on day 35<br>sponsor notes that they were unable to obtain a sample from one HDM<br>samples sent to Glaxo for analysis by HPLC with electrochemical detection |
| necropsy | all | killed following an ip injection of pentobarbitone and exsanguination, all subjected to a full macroscopic examination. |
| organ weights | all | expressed as absolute weight only: brain, pituitary, thymus, heart, lungs, liver, spleen, prostate, kidneys, gonads, adrenals. |
| histopathology processing | | samples of the following organs were immersion fixed in 10% neutral buffered formalin (eyes-Davidsons's, lungs-distended with fixation prior to immersion), paraffin embedded sectioned at 4 microns, H&E stained and examined by LM:<br>adrenals, esophagus, stomach, duodenum, jejunum, ileum, colon, aorta, brain, eyes, femur, heart, kidneys, larynx, liver, lungs, lymph nodes (tracheobronchial, cervical, mesenteric), mammary gland, nasal passages (rostral and caudal nasal cavities), paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, zymbal's gland, ovaries, pancreas, pharynx, pituitary, prostate, cervical spinal cord, spleen, testes with epididymides, thymus, thyroids with parathyroids, tongue, trachea including bifurcation, urinary bladder, uterus and cervix, vagina and gross abnormalities. |
| histopathology examination | gr 1,2,5<br><br>gr 3, 4 | all sampled tissues from main study animals; however, recovery animals only had larynx and tracheal bifurcation examined.<br><br>nasal passages, larynx, trachea (including bifurcation) and lungs |

Results

Mortality: none

Clinical Signs: According to the sponsor the following were considered treatment related: hair loss and brown staining of the head and snout. The hair loss was especially noted in the group 4 and 5 females from week 3 onward. This sign did not regress during the recovery period. Brown staining of the head and snout did regress during recovery period. There was also an apparent treatment related increase in matted fur.

Body Weight Gain: According to the sponsor there was a statistically significant increase in overall body weight gain (weeks 0-5) for the high dose treated males when compared to the vehicle control. Examination of the data does not reveal any clear effect of treatment during the treatment period. During the recovery period, vehicle control and high dose treated females gained less weight than the air control females.

Food Consumption: Recall that this parameter was assessed by cage of five animals. There does not appear to be any consistent effect of treatment. According to the sponsor there were statistically significant increases in overall (week 1-5) food consumption for the mid and high dose treated males when compared to the vehicle control. Low dose treated males were also noted with increased food intake when compared to the vehicle control; however, it was similar to the air control value. The sponsor noted that overall food intake was increased in mid and high dose treated females; however, the difference did not attain statistical significance. During the recovery period the food consumption for high dose treated males was noted as greater than either the air or vehicle controls and for females the high dose and air control groups were noted with greater food consumption than the vehicle control.

Ophthalmoscopy: There was no clear effect of treatment. The sponsor did note a low incidence of capsular opacity in the vehicle control group.

Hematology: Recall that this parameter was assessed only in the animals (10 /sex/group), which were sacrificed after 35 days of treatment. The sponsor's interpretation of the results is based on the statistical significance. According to the sponsor there were no effects of GR43175 treatment on red blood cell parameters. The sponsor notes that the air control group had statistically significantly higher values that the vehicle control for MCHC during week 2. The sponsor points out that at week 2 the high dose treated males had a statistically significant decreased lymphocyte count when compared to the vehicle control; however the value was noted to be similar to the air control value. The sponsor also noted that at week 5 the neutrophil count in the air control female group was lower than that of the of the vehicle control.

A brief examination of the data did not reveal any clearly treatment related changes; however, there were some isolated decreased values for total white blood cells, neutrophils and lymphocytes in mid and high dose treated males during week 5 and very isolated decreases in platelet count for one female in the mid and high dose groups during week 5.

Clinical Chemistry: Recall that this parameter was assessed only in the animals (10 /sex/group), which were sacrificed after 35 days of treatment. The sponsor's interpretation of the results is based on the statistical significance. According to the sponsor the week 5 glucose values for treated males was increased when compared to the vehicle control. The sponsor noted that values for the air controls were even higher and, therefore, dismissed the finding. The sponsor also noted that during week 2 the high dose treated females had significantly higher creatinine values that the vehicle control; however, the difference was considered minimal and not related to treatment. The sponsor noted that during week 2 there were significant increases in sodium values for the mid and high dose treated males and increases in chloride values for all treated males when compared to the vehicle controls. Week 5 values were similar to the vehicle controls. For females the sponsor noted that sodium values were increased in the mid and high dose group at weeks 2 and 5 but only attained significance at week 5. The sponsor noted that the overall difference was small and within expected ranges.

Examination of the individual animal data does not reveal any clearly treatment related alterations ; however, there were some scattered decreased potassium and glucose values in the high dose treated females at week 5.

Urinalysis: This parameter was assessed in only the 10/sex/group sacrificed after 35 days of treatment. According to the sponsor the treated group generally produced less urine during the collection period than the vehicle control groups during both weeks 2 and 5. The sponsor noted that only for the high dose male at week 5 did this attain statistical significance. Examination of the individual animal data revealed an increase in urinary ketones in low, mid and high dose treated males during week 2, which did not persist to week 5, and an apparent decrease in urinary volume in low, mid and high dose females during week 2, which did not persist to week 5.

Macroscopic Pathology: There was an increased incidence of alopecia in the treated animals when compared to the vehicle controls. For females this persisted through the recovery period. There was also an increase in pale subpleural foci of the lung in vehicle control and mid and high dose treated animals, which persisted in the high dose animals (especially the females) through the recovery period.

Organ Weight: Individual animal data was supplied only as absolute organ weight. The sponsor - supplied summary tables had comparisons of absolute organ weight and, for some tissues, tissue weight relative to body weight. The organs for which weight was expressed as a variable of body weight were different for males and females. The sponsor's analysis of the data was based on statistically significant differences. The sponsor pointed out that the mean relative lung weight was decreased in all the low, mid and high dose females compared to the vehicle control. The values were consistent with those of the air control group and, therefore, the sponsor did not attribute this to treatment. The sponsor noted a statistically significant decrease in relative spleen weight in high dose treated males and concluded that the effect was unlikely to be treatment related. The sponsor also noted a statistically significant increase in relative spleen weight for vehicle control females compared to air control females. The sponsor noted that for the recovery group that the brain weight relative to body weight for high dose males was significantly higher than for the vehicle control; however, it was similar to the air control. Also noted were statistically significant differences between the air and vehicle controls for lungs in males and adrenals in females.

Examination of the individual animal data demonstrates possibly treatment related increases in ovary weight in the low, mid and high dose treated group when compared to the air and vehicle controls and some isolated decreases in thymus weight in the mid and high dose treated males compared to the air and vehicle controls. There were no treatment related abnormal pathology associated with these organs (although only the high dose and control groups were examined), therefore, the toxicological significance is questionable. Examination of the individual animal data from the recovery groups did not suggest any persistent effects of treatment.

Histopathology: A copy of the relevant sections of the sponsor-provided summary table follows. The sponsor noted treatment related effects in the larynx and the tracheal bifurcation as discussed below. The only other organ which may have an apparent treatment related effect is the heart, where trace to minimal myocardial mononuclear cells were noted as follows: air control-0//20, vehicle control-0/20 and high dose treated-3/20 (the heart was not examined in the low or mid dose treated animals or the recovery animals).

In order to interpret the treatment related toxicity to the respiratory system it is important to remember that in this study dose was increased by increasing exposure time in the chamber. By design, this varied the exposure time to the vehicle in any vehicle exposed group, adding an additional level of complexity to the analysis. Thus, the vehicle control group and the high dose group have the longest exposure to the vehicle (and for the high dose group, vehicle plus drug) at 60 minutes, followed by the mid dose group at 30 minutes exposure to vehicle (plus drug) and the low dose group had the shortest exposure to vehicle (plus drug) at 15 minutes.

With reference to the larynx, necrosis of the ventral cartilage was noted in all groups exposed to vehicle or vehicle plus drug. This problem occurred in almost all of the vehicle control, mid and high dose treated animals. The lower incidence in the low dose group may be a reflection of the comparatively shorter exposure to the vehicle. Hyperplasia and keratinization of the laryngeal ventral epithelium occurred in the vehicle and treated groups; however, the incidence and severity increased with increasing dose of Ulceration of the ventral epithelium was noted in a single high dose female.

There was a dose related increase in the incidence and severity of hyperplasia and hyperplasia associated with squamous metaplasia of the ventrolateral epithelium in treated animals, which did not occur in the vehicle control group. There was also a treatment related increase in incidence and severity of hyperplasia (mid and high dose groups) and keratinization (high dose group) of the lateral epithelium, which was not present in any control or low dose treated animals. There was also an increase in the incidence and severity of hyperplasia and keratinization of the epithelium of the arytenoid projection in all treated groups.

With reference to the tracheal bifurcation, there was an increased incidence of apparent loss of cilia from the epithelium in the high dose group.

With reference to recovery, recall that only five animals per sex, from the air and vehicle control groups and the high dose treatment group were maintained for a two week recovery period and only the larynx and tracheal bifurcation were examined. After the recovery period necrosis of the ventral cartilage was noted in 8 of 10 vehicle control rats and 10 of 10 high dose treated rats. There was still evidence of increased incidence of hyperplasia of the ventrolateral epithelium (larynx) in the high dose treated group and a treatment related increase in the severity of the hyperplasia of the epithelium of the arytenoid projections (larynx). There was also an apparent treatment related increase in subepithelial mononuclear cells in the arytenoid projection (larynx) .

Plasma Drug Concentration: According to the sponsor, plasma concentrations were similar for males and females, and plasma drug concentrations increased with increasing dose. A reviewer-generated summary table follows.

| Plasma Drug Concentration | | | | |
|---|---|---|---|---|
| group | sex | sample time (min. post dose) | range | mean |
| 2 | male | 13 - 24 | | - |
| | female | 11 - 17 | | - |
| | combined | 11 - 24 | | - |
| 3 | male | 7 - 12 | | 490 ± 232 |
| | female | 8 - 14 | | 444 ± 148 |
| | combined | 7 - 14 | | 467 ± 185 |
| 4 | male | 19 - 27 | | 1106 ± 47 |
| | female | 8 - 23 | | 1065 ± 246 |
| | combined | 8 - 27 | | 1080 ± 168 |
| 5 | male | 12 - 19 | | 2126 ± 340 |
| | female | 10 - 17 | | 1260 ± 204 |
| | combined | 10 - 19 | | 1740 ± 530 |

plasma levels determined using HPLC with electrochemical detection, lower limit of detection 1.0 ng/ml; therefore, nd = <1.0

note three of the five group 2 males and four of the five group 2 females tested had detectable in the plasma

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TABLE 11a

Microscopic pathology incidence summary - rats killed following 35 days of dosing

| | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 | GROUP 5 | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 | GROUP 5 |
|---|---|---|---|---|---|---|---|---|---|---|
| REMOVAL REASON: TERMINAL | | | MALES | | | | | FEMALES | | |
| ANIMALS ON STUDY | 15 | 15 | 10 | 10 | 15 | 15 | 15 | 10 | 10 | 15 |
| ANIMALS COMPLETED | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| NASAL PASSAGES | | | | | | | | | | |
| EXAMINED | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| NO ABNORMALITIES DETECTED | 9 | 8 | 10 | 8 | 8 | 9 | 8 | 10 | 10 | 10 |
| Epithelial and subepithelial mononuclear cells (ventral meatus) (TOTAL) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Minimal | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Loss of cilia in epithelium of middle meatus and turbinates (TOTAL) | 1 | 2 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 0 |
| Minimal | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Moderate | 0 | 2 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| Mucus and inflammatory cells in maxillary sinus | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Atrophy of olfactory epithelium (dorsal meatus) (TOTAL) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Minimal | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Attenuation of olfactory epithelium (middle part of nasal septum) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Atrophy of lateral nasal glands (TOTAL) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Moderate | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| LARYNX | | | | | | | | | | |
| EXAMINED | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| NO ABNORMALITIES DETECTED | 6 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 0 |
| Necrosis of ventral cartilage | 0 | 9 | 7 | 9 | 10 | 0 | 8 | 2 | 9 | 10 |
| Ulceration of ventral epithelium | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hyperplasia of ventral epithelium (TOTAL) | 0 | 4 | 6 | 8 | 8 | 0 | 3 | 4 | 7 | 9 |
| Minimal | 0 | 1 | 6 | 1 | 1 | 0 | 3 | 4 | 6 | 1 |
| Moderate | 0 | 3 | 0 | 5 | 6 | 0 | 0 | 0 | 1 | 8 |
| Marked | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| ANIMALS ON STUDY | 15 | 15 | 10 | 10 | 15 | 15 | 15 | 10 | 10 | 15 |
| ANIMALS COMPLETED | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| LARYNX | (CONTINUED) | | | | | | | | | |
| Keratinisation of ventral epithelium (TOTAL) | 0 | 2 | 0 | 2 | 4 | 0 | 0 | 0 | 0 | 4 |
| Trace | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 2 |
| Minimal | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 2 |
| Inflammatory cells in submucosa around ventral pouch (TOTAL) | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 0 |
| Minimal | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| Moderate | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Hyperplasia of ventrolateral epithelium (TOTAL) | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 2 | 0 | 0 |
| Minimal | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 2 | 0 | 0 |
| Hyperplasia and squamous metaplasia of ventrolateral epithelium (TOTAL) | 0 | 0 | 2 | 1 | 7 | 0 | 0 | 0 | 1 | 7 |
| Minimal | 0 | 0 | 2 | 1 | 4 | 0 | 0 | 0 | 1 | 2 |
| Moderate | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 5 |
| Hyperplasia of lateral epithelium (TOTAL) | 0 | 0 | 0 | 4 | 7 | 0 | 0 | 0 | 3 | 5 |
| Minimal | 0 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 3 | 2 |
| Moderate | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| Keratinisation of lateral epithelium (TOTAL) | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 3 |
| Trace | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Minimal | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 2 |
| Hyperplasia of epithelium of arytenoid projections (TOTAL) | 4 | 2 | 9 | 9 | 9 | 5 | 5 | 9 | 7 | 9 |
| Minimal | 2 | 2 | 8 | 5 | 5 | 5 | 5 | 7 | 0 | 4 |
| Moderate | 2 | 0 | 1 | 4 | 4 | 0 | 0 | 2 | 7 | 5 |
| Keratinisation of epithelium of arytenoid projections (TOTAL) | 1 | 0 | 2 | 4 | 5 | 0 | 0 | 0 | 6 | 8 |
| Trace | 1 | 0 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| Minimal | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 6 | 5 |
| Moderate | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 3 |

(Microscopic pathology incidence summary - rats killed following 35 days of dosing - continued)

| | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 | GROUP 5 | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 | GROUP 5 |
|---|---|---|---|---|---|---|---|---|---|---|
| REMOVAL REASON: TERMINAL | | | MALES | | | | | FEMALES | | |
| ANIMALS ON STUDY | 15 | 15 | 10 | 10 | 15 | 15 | 15 | 10 | 10 | 15 |
| ANIMALS COMPLETED | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| LARYNX (CONTINUED) | | | | | | | | | | |
| Subepithelial mononuclear cells in arytenoid projections (TOTAL) | 0 | 0 | 5 | 2 | 0 | 1 | 0 | 1 | 1 | 0 |
| Minimal | 0 | 0 | 5 | 2 | 0 | 1 | 0 | 1 | 1 | 0 |
| Mucus and inflammatory cells in lumen. | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Muscle fibre degeneration with mononuclear cells (TOTAL) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Minimal | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| TRACHEA | | | | | | | | | | |
| EXAMINED | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| NO ABNORMALITIES DETECTED | 10 | 10 | 8 | 10 | 10 | 10 | 9 | 10 | 10 | 10 |
| Inflammatory cells in the lamina propria (TOTAL) | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Trace | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Minimal | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TRACHEAL BIFURCATION | | | | | | | | | | |
| EXAMINED | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 10 | 10 | 9 |
| MISSING | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 |
| NO ABNORMALITIES DETECTED | 6 | 8 | 7 | 10 | 7 | 6 | 8 | 7 | 10 | 5 |
| Mononuclear cells in lamina propria (TOTAL) | 4 | 2 | 3 | 0 | 0 | 2 | 1 | 2 | 0 | 1 |
| Trace | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Minimal | 2 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |
| Moderate | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Apparent loss of cilia from epithelium | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 0 | 3 |
| LUNGS | | | | | | | | | | |
| EXAMINED | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| MISSING | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NO ABNORMALITIES DETECTED | 1 | 1 | 3 | 1 | 2 | 7 | 3 | 7 | 4 | 5 |
| ANIMALS ON STUDY | 15 | 15 | 10 | 10 | 15 | 15 | 15 | 10 | 10 | 15 |
| ANIMALS COMPLETED | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| LUNGS (CONTINUED) | | | | | | | | | | |
| Pneumonitis (TOTAL) | 5 | 6 | 6 | 8 | 8 | 2 | 5 | 2 | 0 | 4 |
| Trace | 1 | 0 | 2 | 1 | 2 | 2 | 3 | 2 | 0 | 1 |
| Minimal | 2 | 4 | 3 | 6 | 5 | 0 | 2 | 0 | 0 | 3 |
| Moderate | 2 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Pneumonitis with prominent alveolar macrophages and epithelialisation (TOTAL) | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Minimal | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Moderate | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Perivascular lymphoid infiltration (TOTAL) | 8 | 9 | 4 | 8 | 7 | 2 | 5 | 1 | 4 | 3 |
| Trace | 2 | 1 | 0 | 1 | 0 | 2 | 2 | 0 | 1 | 0 |
| Minimal | 1 | 4 | 3 | 6 | 5 | 0 | 2 | 1 | 3 | 3 |
| Moderate | 5 | 4 | 1 | 1 | 2 | 0 | 1 | 0 | 0 | 0 |
| Prominent BALT | 2 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Subpleural alveolar macrophages | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Arterial medial hypertrophy | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 2 | 0 |
| Perivascular inflammatory cells (TOTAL) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Trace | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| AORTA | | | | | | | | | | |
| EXAMINED | 10 | 10 | 0 | 0 | 10 | 10 | 10 | 0 | 0 | 10 |
| NO ABNORMALITIES DETECTED | 10 | 10 | 0 | 0 | 10 | 10 | 10 | 0 | 0 | 10 |
| HEART | | | | | | | | | | |
| EXAMINED | 10 | 10 | 0 | 0 | 10 | 10 | 10 | 0 | 0 | 10 |
| NO ABNORMALITIES DETECTED | 10 | 10 | 0 | 0 | 7 | 10 | 9 | 0 | 0 | 10 |
| Myocardial mononuclear cells (TOTAL) | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Trace | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Minimal | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |

Microscopic pathology incidence summary - rats killed following 14 days of withdrawal

| REMOVAL REASON: RECOVERY | GROUP 1 | GROUP 2 | GROUP 5 | GROUP 1 | GROUP 2 | GROUP 5 |
|---|---|---|---|---|---|---|
| | -------- MALES -------- | | | -------- FEMALES -------- | | |
| ANIMALS ON STUDY | 15 | 15 | 15 | 15 | 15 | 15 |
| ANIMALS COMPLETED | 5 | 5 | 5 | 5 | 5 | 5 |
| LARYNX | | | | | | |
| EXAMINED | 5 | 5 | 5 | 5 | 5 | 5 |
| NO ABNORMALITIES DETECTED | 2 | 0 | 0 | 2 | 1 | 0 |
| Necrosis of ventral cartilage | 0 | 5 | 5 | 0 | 3 | 5 |
| Hyperplasia of ventral epithelium (TOTAL) | 0 | 2 | 0 | 0 | 0 | 0 |
| Minimal | 0 | 2 | 0 | 0 | 0 | 0 |
| Inflammatory cells in submucosa around ventral pouch (TOTAL) | 0 | 1 | 0 | 0 | 0 | 0 |
| Minimal | 0 | 1 | 0 | 0 | 0 | 0 |
| Hyperplasia and squamous metaplasia of ventral pouch epithelium (TOTAL) | 1 | 1 | 0 | 0 | 0 | 0 |
| Minimal | 1 | 1 | 0 | 0 | 0 | 0 |
| Inflammatory cells and foreign material within ventral pouch | 0 | 1 | 0 | 0 | 0 | 0 |
| Hyperplasia of ventrolateral epithelium (TOTAL) | 1 | 0 | 1 | 0 | 1 | 2 |
| Minimal | 1 | 0 | 1 | 0 | 1 | 2 |
| Hyperplasia of epithelium of arytenoid projections (TOTAL) | 2 | 3 | 3 | 3 | 2 | 2 |
| Minimal | 2 | 3 | 2 | 3 | 2 | 1 |
| Moderate | 0 | 0 | 1 | 0 | 0 | 1 |
| Subepithelial mononuclear cells in arytenoid projections (TOTAL) | 0 | 0 | 1 | 0 | 0 | 1 |
| Minimal | 0 | 0 | 1 | 0 | 0 | 1 |
| Mucus and inflammatory cells in lumen | 1 | 0 | 1 | 0 | 2 | 0 |
| TRACHEAL BIFURCATION | | | | | | |
| EXAMINED | 5 | 5 | 5 | 5 | 5 | 5 |
| NO ABNORMALITIES DETECTED | 4 | 3 | 3 | 4 | 2 | 3 |
| Mononuclear cells in lamina propria (TOTAL) | 1 | 2 | 2 | 0 | 2 | 1 |
| Trace | 0 | 1 | 0 | 0 | 1 | 1 |
| Minimal | 1 | 1 | 2 | 0 | 1 | 0 |
| TRACHEAL BIFURCATION (CONTINUED) | | | | | | |
| Apparent loss of cilia from epithelium | 0 | 0 | 0 | 1 | 1 | 1 |



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**Preliminary**

**Evaluation in Guinea Pigs of Potential Skin Irritancy Resulting from Single Occluded Applications**

Test Facility: not stated
non-GLP (notable exceptions - see appended page)

Test Formulati

were made into 50% (base equivalent) w/v solutions in water for irrigation. (batch # C668/265/1) was made into a 10% (base equivalent) w/v solution in water for irrigation. Water for irrigation was the vehicle control and sodium lauryl sulphate (10% w/v in water for irrigation) was the positive control.

Animals: Female Dunkin Hartley derived guinea pigs, weighing 250-300g.

Protocol: Both flanks of each animal were clipped and depilated. One flank of each animal was abraded with a hypodermic needle. Animals were divided into five treatment groups of five per group. The appropriate test formulation was applied to both the abraded and the intact flank as 0.25 ml per site on a 2x2 cm square of a double thickness of a product called Postslip Paper. The administration sites were occluded for approximately 21 hours with polyethylene sheeting fastened with surgical adhesive tape (Micropore) and adhesive bandage (Elastoplast).

Observations: The test formulation application sites were examined at approximately 24 and 48 hours after application. Reaction scores for each animal were determined based on the following scoring system.

| Grading of Irritancy Reactions | |
|---|---|
| observation | score |
| no reaction | 0 |
| slight erythema and/or edema, or superficial eschar formation | 1 |
| well defined erythema and/or edema | 2 |
| moderate to severe erythema and/or edema | 3 |
| as 3 plus slight to moderate necrosis | 4 |
| as 3 plus marked necrosis | 5 |

Group mean irritancy scores were calculated and classified according to the following system:

| Irritancy Classification | |
|---|---|
| group mean score | classification |
| 0 - 0.9 | negligible |
| 1.0 - 1.9 | slight |
| 2.0 - 3.4 | moderate |
| 3.5 - 5.0 | severe |

Skin fold thickness at the application site was also assessed. On the last day of the study, the application sites were removed, fixed in 10% buffered formalin, embedded in paraffin; however, microscopic examination was not carried out.

Results

General: According to the sponsor there was "no general loss of condition" and there was no effect of treatment on body weights.

Irritancy:

| Irritancy Scores and Rating | | | | | | | |
|---|---|---|---|---|---|---|---|
| group | time (hours) | range of individual scores | | group mean irritancy scores | | irritancy rating | |
| | | intact | abraded | intact | abraded | intact | abraded |
| water control | 24 | 0 | 0 -1 | 0.0 | 0.4 | negligible | negligible |
| | 48 | 0 | 0 - 1 | 0.0 | 0.2 | negligible | negligible |
| 50% GR43175N | 24 | 0 -1 | 1 - 4 | 0.2 | 3.4 | negligible | moderate |
| | 48 | 0 | 1 - 4 | 0.0 | 2.4 | negligible | moderate |
| 50% GR43175D | 24 | 0 - 1 | 1 - 4 | 0.2 | 2.2 | negligible | moderate |
| | 48 | 0 | 0 - 4 | 0.0 | 2.0 | negligible | moderate |
| 10% GR43175C | 24 | 0 - 1 | 0 - 1 | 0.6 | 0.6 | negligible | negligible |
| | 48 | 0 -1 | 0 - 1 | 0.2 | 0.2 | negligible | negligible |
| 10% sodium lauryl sulphate | 24 | 2 - 4 | 2 - 4 | 2.8 | 3.0 | moderate | moderate |
| | 48 | 3 - 4 | 3 - 4 | 3.4 | 3.4 | moderate | moderate |

| Skinfold Thickness | | | | | |
|---|---|---|---|---|---|
| group | time (hours) | range of individual scores | | group mean skinfold thickness (mm) | |
| | | intact | abraded | intact | abraded |
| water control | pre | 2.0 - 2.5 | 2.1 - 2.6 | 2.16 | 2.38 |
| | 24 | 2.4 - 2.6 | 2.7 - 3.0 | 2.48 | 2.84 |
| | 48 | 2.2 - 2.5 | 2.4 - 3.0 | 2.30 | 2.70 |
| 50% GR43175N | pre | 2.3 - 2.7 | 2.5 - 2.7 | 2.44 | 2.60 |
| | 24 | 2.5 - 2.9 | 2.9 - 4.1 | 2.68* | 3.56* |
| | 48 | 2.5 - 2.8 | 2.9 - 4.0 | 2.66 | 3.58* |
| 50% GR43175D | pre | 2.0 - 2.5 | 2.3 - 2.6 | 2.26 | 2.46 |
| | 24 | 2.4 - 2.6 | 2.9 - 4.4 | 2.48 | 3.38 |
| | 48 | 2.1 - 2.5 | 2.8 -3.7 | 2.32 | 3.20 |
| 10% GR43175C | pre | 2.2 - 2.4 | 2.2 - 2.6 | 2.30 | 2.42 |
| | 24 | 2.4 - 3.1 | 2.8 - 3.0 | 2.70 | 2.86 |
| | 48 | 2.3 - 2.8 | 2.5 - 2.9 | 2.48 | 2.68 |
| 10% sodium lauryl sulphate | pre | 2.3 - 2.5 | 2.2 - 2.7 | 2.38 | 2.48 |
| | 24 | 2.8 - 3.3 | 2.9 - 3.6 | 3.06* | 3.28* |
| | 48 | 3.5 - 4.9 | 3.8 - 4.4 | 4.18* | 4.18* |

* - group significantly different ($p < 0.05$) from control

## GLP COMPLIANCE STATEMENT

Report No: WPT/87/019

Type of Study: Preliminary evaluation in guinea pigs of potential skin irritancy resulting from single occluded applications
(Study No. G11179)



This preliminary study was conducted in July 1986 in accordance with the principles of good laboratory practice. However, it did not comply in all respects with the FDA Good Laboratory Practice Regulations (FDA, Title 21 CFR Part 58, US Federal Register, 22 December 1978 and subsequent amendment, 11 April 1980) or the OECD Principles of Good Laboratory Practice (ISBN 92-64-12367-9, Paris 1982). The instances of non-compliance are classified below according to the relevant subpart of the FDA GLP Regulations.

Subpart B

1) There was no independent monitoring of the various phases of the study by the Quality Assurance Unit, nor was the final report audited.

2) The study was not included on the Master Schedule Sheet kept by the Quality Assurance Unit.

Subpart F

1) The test article/carrier mixtures were not analysed to determine test article concentration.

2) The dry stability of the test articles GR43175D & GR43175N was not determined although their stability in a mixture with the carrier was determined.

Subpart G

1) The written protocol for the study did not contain all the information specified in subsection 58.120.

Subpart J

1) The final report of the study does not contain all the information specified in subsection 58.185. In particular no analytical data relating to the test articles or test article/carrier mixtures are included.

Despite these deviations from the particular requirements of the regulations, there is no reason to believe that the scientific integrity of the study was compromised in any way.

Study Director

**Further Preliminary Evaluation in Guinea Pigs of Potential Skin Irritancy Resulting from Single Occluded Applications.**

Test Facility: not stated
non-GLP (notable exceptions - see appended page)

Test Formulations:

were made into a 10% (base equivalent) w/v solution in water for irrigation. Water for irrigation was the vehicle control and sodium lauryl sulphate (10% w/v in water for irrigation) was the positive control. The pH of each solution was determined and are listed as follows: water for irrigation (vehicle) - 4.70, 10% w/v - 4.50, 10% w/v - 3.43, 10% w/v - 4.68, and 10% w/v sodium lauryl sulphate - 8.17.

Animals: Female Dunkin Hartley derived guinea pigs, weighing 250-300g.

Protocol: Both flanks of each animal were clipped and depilated. One flank of each animal was abraded with a hypodermic needle. Animals were divided into five treatment groups of five per group. The appropriate test formulation was applied to both the abraded and the intact flank as 0.25 ml per site on a 2x2 cm square of a double thickness of a product called Postslip Paper. The administration sites were occluded for approximately 21 hours with polyethylene sheeting fastened with surgical adhesive tape (Micropore) and adhesive bandage (Elastoplast).

Observations: The test formulation application sites were examined at approximately 24 and 48 hours after application. Reaction scores for each animal were determined based on the following scoring system.

| Grading of Irritancy Reactions | |
|---|---|
| observation | score |
| no reaction | 0 |
| slight erythema and/or edema, or superficial eschar formation | 1 |
| well defined erythema and/or edema | 2 |
| moderate to severe erythema and/or edema | 3 |
| as 3 plus slight to moderate necrosis | 4 |
| as 3 plus marked necrosis | 5 |

Group mean irritancy scores were calculated and classified according to the following system:

| Irritancy Classification | |
|---|---|
| group mean score | classification |
| 0 - 0.9 | negligible |
| 1.0 - 1.9 | slight |
| 2.0 - 3.4 | moderate |
| 3.5 - 5.0 | severe |

Skin fold thickness at the application site was also assessed. On the last day of the study, the application sites were removed, fixed in 10% buffered formalin, embedded in paraffin; however, microscopic examination was not carried out.

Results

General: One animal treated with was sacrificed prior to the initiation of treatment. For all of the other animals the sponsor states that there was "no general loss of condition" and there was no effect of treatment on body weights.

Irritancy:

| Irritancy Scores and Rating | | | | | | | |
|---|---|---|---|---|---|---|---|
| group | time (hours) | range of individual scores | | group mean irritancy scores | | irritancy rating | |
| | | intact | abraded | intact | abraded | intact | abraded |
| water control | 24 | 0 | 0 - 1 | 0.0 | 0.6 | negligible | negligible |
| | 48 | 0 | 0 - 1 | 0.0 | 0.4 | negligible | negligible |
| 10% GR43175N | 24 | 0 - 1 | 0 - 1 | 0.3 | 0.6 | negligible | negligible |
| | 48 | 0 | 0 - 1 | 0.0 | 0.6 | negligible | negligible |
| 10% GR43175D | 24 | 0 - 1 | 1 | 0.4 | 1.0 | negligible | slight |
| | 48 | 0 | 0 - 1 | 0.0 | 0.8 | negligible | negligible |
| 10% GR43175C | 24 | 0 - 1 | 0 - 1 | 0.2 | 0.8 | negligible | negligible |
| | 48 | 0 | 0 - 1 | 0.0 | 0.4 | negligible | negligible |
| 10% sodium lauryl sulphate | 24 | 2 - 4 | 3 - 4 | 3.6 | 3.8 | severe | severe |
| | 48 | 3 - 5 | 4 - 5 | 4.2 | 4.8 | severe | severe |

| Skinfold Thickness | | | | | |
|---|---|---|---|---|---|
| group | time (hours) | range of individual scores | | group mean skinfold thickness (mm) | |
| | | intact | abraded | intact | abraded |
| water control | pre | 1.9 - 2.3 | 1.8 - 2.4 | 2.16 | 2.18 |
| | 24 | 2.3 - 2.6 | 2.7 - 3.3 | 2.46 | 2.88 |
| | 48 | 2.0 - 2.6 | 2.3 - 3.2 | 2.30 | 2.70 |
| 10% GR43175N | pre | 2.0 - 2.4 | 2.0 - 2.5 | 2.18 | 2.30 |
| | 24 | 2.4 - 2.8 | 2.8 - 3.2 | 2.55 | 3.02 |
| | 48 | 2.4 - 2.8 | 2.8 - 3.1 | 2.47 | 2.88 |
| 10% GR43175D | pre | 1.8 - 2.6 | 2.0 - 2.6 | 2.16 | 2.28 |
| | 24 | 2.2 -2.6 | 3.0 - 3.1 | 2.48 | 3.06 |
| | 48 | 2.0 -2.6 | 2.7 - 3.0 | 2.32 | 2.86 |
| 10% GR43175C | pre | 2.1 - 2.3 | 2.2 - 2.5 | 2.20 | 2.36 |
| | 24 | 2.4 - 2.8 | 2.6 - 3.3 | 2.54 | 2.90 |
| | 48 | 2.3 - 2.6 | 2.6 - 3.1 | 2.42 | 2.80 |
| 10% sodium lauryl sulfate | pre | 1.9 - 2.5 | 2.0 - 2.6 | 2.32 | 2.44 |
| | 24 | 2.3 - 4.3 | 3.0 - 4.3 | 3.30* | 3.66* |
| | 48 | 3.5 - 4.5 | 3.9 - 5.7 | 4.06* | 5.08* |

## GLP COMPLIANCE STATEMENT

Report No: WPT/87/050

Type of Study: Further preliminary evaluation in guinea-pigs of potential skin irritancy resulting from single occluded applications
(Study No. G11191)

This preliminary study was conducted in July 1986 in accordance with the principles of good laboratory practice. However, it did not comply in all respects with the FDA Good Laboratory Practice Regulations (FDA, Title 21 CFR Part 58, US Federal Register, 22 December 1978 and subsequent amendment, 11 April 1980) or the OECD Principles of Good Laboratory Practice (ISBN 92-64-12367-9, Paris 1982). The instances of non-compliance are classified below according to the relevant subpart of the FDA GLP Regulations.

Subpart B

1) There was no independent monitoring of the various phases of the study by the Quality Assurance Unit, nor was the final report audited.

2) The study was not included on the Master Schedule Sheet kept by the Quality Assurance Unit.

Subpart F

1) The test article/carrier mixtures were not analysed to determine test article concentration.

2) The dry stability of the test articles GR43175D & GR43175N was not determined although their stability in a mixture with the carrier was determined.

Subpart G

1) The written protocol for the study did not contain all the information specified in subsection 58.120.

Subpart J

1) The final report of the study does not contain all the information specified in subsection 58.185. In particular no analytical data relating to the test articles or test article/carrier mixtures are included.

Despite these deviations from the particular requirements of the regulations, there is no reason to believe that the scientific integrity of the study was compromised in any way.

Study Director

**Summary and Evaluation**

This NDA was submitted to support the intranasal administration of Imitrex (sumatriptan, , for treatment of acute migraine. Imitrex is already marketed by the subcutaneous route (NDA 20-080) and the oral route (NDA 20-132) for treatment of acute migraine. The pharmacology and preclinical toxicology of sumatriptan by the oral and subcutaneous routes has been presented and reviewed for those NDAs. This review will focus on intranasal toxicity and irritancy and, to a lesser extent, ocular irritancy, and skin irritancy.

The clinical formulations of intranasal Imitrex proposed for marketing will include the following concentrations o in a phosphate buffer:

a. 5 mg(base)/0.1 ml or 50 mg(base)/ml
b. 10 mg(base)/0.1 ml or 100 mg(base)/ml
c. 20 mg(base)/0.1 ml or 200 mg(base)/ml

The proposed recommended human dose is a single 0.1 ml spray of the 200 mg(base)/ml solution (i.e., 20 mg(base) into one nostril). The dosing device is a single use (spray) unit. If the headache returns after the initial treatment, this dose can be repeated after two hours, for a total daily dose of up to 40 mg. Apparently, lower dose concentration solutions will be available.

Note that the proposed clinical formulation is
The marketed oral and injectable forms of sumatriptan contain

Pharmacokinetic Studies:

Toxicity studies conducted to support the injectable (NDA 20-080) and the oral (NDA 20-132) formulations could be used to support the systemic toxicity of intranasally administered sumatriptan, provided that the sponsor has shown that the plasma metabolic profiles for sumatriptan are similar among the different routes of administration, and that the metabolic profiles from animals treated by the nasal route are similar to that produced by the humans treated by the nasal route. The sponsor has not studied metabolism in humans after intranasal administration of sumatriptan. The sponsor has measured the major metabolite produced in humans after oral or subcutaneous administratio
of the parent compound) and measured it in urine and serum after intranasal dosing with
According to the 12/29/95 Biopharmaceutics Review of this NDA, after intranasal administration, 42% of the dose is excreted in urine as and, in the serum, this metabolite is present in four times greater amounts than the parent compound.

The sponsor has provided pharmacokinetic studies comparing intranasal and intravenous administration of . sing the clinical formulation, one in Sprague Dawley rats and one in beagle dogs (see review pages 1-10). It should be noted that in this study intranasal administration was carried out with a displacement pipet, rather than a spray. In the intranasal toxicity studies, dosing was carried out by spray. This difference could alter some of the absorption patterns, possibly providing more of an oral component. In dogs bioavailability (based on AUC ) of the parent compound after intranasal administration was approximately 50% in both males and females. In rats bioavailability (based on $AUC_{0\text{-}6\ hours}$) of the parent compound after intranasal administration was approximately was approximately 30% for both males and females.

The pharmacokinetic studies indicate that the drug is highly metabolized. The sponsor provided an assessment of the urine metabolite profile in both rats and dogs after intravenous and intranasal administration of radiolabelec . The high degree of metabolism of the parent compound is reflected in the urine profiles. For rats
comprise the majority of the radioactivity excreted in the urine with up to five additional unidentified areas of radioactivity, one of which is major, after intranasal administration. After intravenous administration, the pattern is similar, among the major components. For dogs, up to nine regions of radioactivity were identified in the urine after intravenous or intranasal administration of radiolabeled . The urinary metabolite profiles seem to be qualitatively similar between the two routes. The majority of radioactivity was present as the parent compound and

Intranasal Irritancy Studies, Inhalation Toxicity Studies and Ocular Irritation Study

These studies are categorized and summarized below, according to the drug formulation employed. Please refer to the reviewer-generated toxicity summary tables #1, 2a, 2b and 3, which appear on pages 66-67. Also note that as part of each intranasal irritancy study, or inhalation toxicity study, the sponsor measured basic toxicokinetic parameters. The toxicokinetic data is presented within the review of each study.

Studies Using the Buffered Clinical Formulation:

Study # 13901 was a 14 day intranasal irritancy study in dogs using the proposed clinical formulation (hemisulfate salt in buffer). This was the only intranasal irritancy study carried out with the proposed formulation (see review pages 11 - 15). In this study 8 dogs (4/sex) were treated with the test formulation and the results compared (unblinded) to 8 control animals (4/sex). On the first day of treatment three (100 microliter) sprays of the clinical formulation were made into each nostril, with the plan that this would be done twice per day for 14 or 15 days. Due to the severe clinical signs (trembling, splayed hindlimbs, unsteady gait, vocalization, subdued behavior and pupil dilation) many of which were still present eight hours post dose, no further doses were given on day one and the dosing regimen for the rest of the study was altered, such that each dog received three (100 microliter) sprays into the right nostril only, twice per day for the remaining 13 or 14 days. From day two onward treatment related clinical signs consisted of salivation, subdued behavior, pupil dilation, trembling, splayed hindlimbs, unsteady gait and vocalization. The doses administered were: 120 mg in one session for day 1 and 60 mg, b.i.d. for days 2-14. All animals survived the study. Histopathologic examination of the nasal cavity and respiratory organs (specifically, trachea, lungs, larynx and oropharynx (epiglottis/pharynx), tracheobronchial lymph node and nasal passage) were carried out.

The unblinded histopathology reading was carried out by a contract organization (different from the two which carried out the (1) in-life portion and necropsy and (2) tissue processing). There was no mention in the pathology report that dosing for each animal was confined to the right nostril only. The report does state that anterior and posterior nasal cavities were studied. According to the pathologists report there were no treatment related findings: "A variety of very minor degenerative and inflammatory lesions were observed. These fell within the range for dogs of this age and strain, and were considered unrelated to treatment." Examination of the data reveals that one of the eight treated dogs was noted with focal squamous metaplasia of the bronchial epithelium of the lungs (grade 1). No other studies intranasal studies have been carried out with the test formulation, so this one finding cannot be confirmed or dismissed. The sponsor did supply (1/28/96), at the request of the reviewer, the procedure for the sectioning of the nasal cavity and respiratory organs. From the information provided in the original submission and the 1/28/96 amendment , it is not clear whether local irritation to the nasal cavity and the respiratory organs was adequately assessed in this study. Generally in the nasal cavity and parts of the respiratory system, the highly specific regional histopathology changes dramatically over small distances. Descriptions of the positions of the cuts, which determine the tissue blocks for histopathology, in isolation do not provide assurance that histopathological examinations of the nasal cavity and respiratory organs included all of the appropriate representative types of epithelium and tissue (e.g., the squamous, the respiratory and the olfactory epithelium of the nasal cavity and transition zones).

The only other toxicity study carried out with the proposed clinical formulation (hemisulfate salt in buffer) was study # L20270, an acute eye irritation study in rabbits (see review pages 16 - 18). In this study the right eye of one rabbit was treated with a 0.01 ml dose of the 200 mg(base)/ml (clinical formulation) and the right eye of three additional rabbits were treated with 0.1 ml of the clinical formulation. The single dose per animal was administered as a drop into the conjunctival sac, followed an assessment of initial pain reaction and classification of the ocular irritancy at set time points for up to 72 hours after dosing. The low dose treated rabbit (0.01 ml) was noted with an initial pain reaction of 'no initial pain' and only redness of the conjunctiva at the one hour observation period. For the three rabbits treated with the higher dose of the test formulation (0.1 ml), each animals was noted with an initial pain reaction of 'slight initial pain,' and two of the three treated were noted only with redness of the conjunctiva at the one hour observation period. For both treatment groups, according to the rating system and classification system employed for the study, this was a very slight reaction and the resulting classification for the clinical formulation would be as a 'non-irritant.'

It is not clear whether this study was adequate by design to address this issue of ocular irritation in a definitive way. The study was very small with a total exposure of four eyes. Exposure was achieved by placing a stated volume of the test formulation into the conjunctiva; however, since the proposed marketed device is a nasal spray, a more appropriate test might involve a spray to the entire external surface of the eye. This test is based on appearance (though the cornea is examined under UV light with the aid of fluorescein) and not pathology. It may be possible that milder or more subtle problems may be overlooked.

Studies Using A Formulation:

Two additional nasal irritancy studies were carried out with a variant of the clinical formulation, which contained the formulation. According to the sponsor the development of the unbuffered formulation was abandoned because the solution became significantly more and storage.

The first study, study #D12787, was a 13 week intranasal tolerance study in the dog (see review pages 19 - 25). In this study beagle dogs (4/sex/group) were treated daily for 13 weeks with either the vehicle control, or the test formulation at concentrations of 200 mg(base)/ml or 400 mg(base)/ml. The daily dosing regimen consisted of three 100 microliter sprays per nostril per session for two daily sessions. The low dose group received 240 mg(base) per day (120 per session) and the high dose received 480 mg(base) per day (240 per session). Thus, animals were exposed, twice per day, to concentrations which were the same as that in the proposed clinical formulation in the low dose group and twice the concentration in the high dose group. Treatment related clinical signs included mydriasis and salivation. There was also a notation of superficial corneal opacities in one low dose treated female and two high dose treated females on day 6. This resulted in a change in the protocol to cover the eyes during subsequent dosing. This finding was not discussed further, and there is no indication of follow-up examination of the eyes. This finding may be similar or related to the corneal opacity seen with oral or subcutaneous dosing of dogs with sumatriptan succinate (see Addendum to Pharmacologist's Review for NDA 20-132, dated 1/13/95), or an independent finding possibly associated with contamination of the eye with some of the test formulation spray.

Histopathology was carried out only on the larynx with pharynx, lung with mainstem bronchi, retropharyngeal and bronchial lymph nodes, nasal passages, tongue, trachea and bifurcation, and gross lesions. According to the sponsor: "There were no significant findings in the nasal cavities or other parts of the respiratory tract." The size of the treatment groups was fairly small and when the findings in males and females were combined there were several possibly treatment related findings in the respiratory organs and nasal cavity, which included: fibrosing alveolitis, bronchitis and granuloma (lung). In the high dose group there were also isolated incidences of minimal lymphoid hyperplasia of the larynx, minimal lymphoid hyperplasia of the nasal passages (respiratory region). There was an isolated incidence of slight hyperplasia of the bronchial lymph node in the low dose group.

Again, these low incidence findings cannot be readily confirmed or dismissed, since this was the only intranasal study in dogs carried out with this formulation. Again, from the information provided in the original submission and the 1/28/96 amendment, it is not clear whether local irritation to the nasal cavity and the respiratory organs was adequately assessed in this study.

The second of the two studies using the unbuffered formulation was study # P11224, a 14 day intranasal irritancy study in cynomolgus monkeys (see review page 26 - 31). This was the only study carried out in cynomolgus monkeys submitted to this NDA. In this study monkeys (2/sex/group) were treated daily for 13 consecutive days with either a vehicle control or the test formulation at concentrations of 200 mg(base)/ml or 400 mg(base)/ml. The daily dosing regimen consisted of one 100 microliter spray per nostril per session for four daily sessions. The low dose group received 160 mg(base) per day (40 per session) and the high dose received 320 mg(base) per day (80 per session). Thus, animals were exposed, four times per day, to concentrations which were the same as that in the proposed clinical formulation in the low dose group and twice the concentration in the high dose group. Treatment related clinical signs included: salivation, vomiting, retching, glazed appearance of eyes, nasal discharge, wiping and rubbing mouth. The sponsor altered the planned dosing regimen on the last day of treatment, so that all animals were subjected to one intranasal dosing session and then, one male and one

female from the low and high dose groups, were subjected to repeated intravenous injections of the test formulation to determine if systemic administration of the test formulation could produce salivation, which it could.

Histopathology was carried out on the nasal passages, pharynx, larynx, heart, trachea, carina, lungs and bronchi. According to the sponsor no macroscopic lesions or histopathologic findings were attributable to The sponsor specifically states: "There were, in particular, no histopathological changes in the nasal passages." Recall that this study has four animals per treatment group; therefore, single findings cannot be easily dismissed. One high dose female was noted with pulmonary fibrosis and left pleural adhesion. One high dose female was noted with patchy discolorations of the tongue which were not examined for histopathology.

This study was problematic. There were several related issues concerning the stability of the test formulation. The study report states that the day 14 samples were returned to the sponsor for analysis. The data presented for this analysis was for solutions of nominal concentrations of 5, 10 , 20 , 40 , 80 and 100 mg(base)/ml rather than the formulations used in this study which were 200 and 400 mg(base)/ml. In addition, according to the study protocol each day fresh solutions of test substance were added to the residue in the nasal spray bottle from the previous days's dosing. This could potentially lead to a problem of less potent, more contaminated test solutions being used as the study progressed. Finally, this study was a small study (4/sex/group) and the only study in monkeys submitted to this NDA and as such, must stand alone. Because of this, it is difficult to dismiss findings seen only in single animals. Again, from the information provided in the original submission and the 1/28/96 amendment , it is not clear whether local irritation to the nasal cavity and the respiratory organs was adequately assessed in this study.

Studies Using Sweetened Formulation:

In the original IND for this drug, the sponsor referred to a study

The sponsor did carry out and complete an additional intranasal irritancy study with the sweetened formulation. This study, study #D13342, a thirteen week intranasal irritancy study in beagle dogs employed a test formulation containing the hemisulfate salt of in water containing 2% sodium saccharin (see review page 32 - 38). In this study beagle dogs (4/sex) were treated daily for 13 weeks with either a vehicle control or the test formulation at concentrations of 200 or 400 mg(base)/ml. The daily dosing regimen consisted of three 100 microliter sprays per nostril per session for two daily sessions. The low dose group received 240 mg(base) per day (120 per session) and the high dose received 480 mg(base) per day (240 per session). Thus, animals were exposed, twice per day, to concentrations which were the same as that in the proposed clinical formulation in the low dose group and twice the concentration in the high dose group. Treatment related clinical signs included: mydriasis, salivation, high pitched vocalization and less frequently, vomiting, lachrymation and ocular opacity.

Histopathology was carried out on the larynx with pharynx, lungs (with mainstem bronchi), lymph nodes (retropharyngeal and bronchial), nasal passages, tongue, trachea and bifurcation. The sponsor states: "There is no evidence of a gross lesion attributable to the test material administration." However, according to the protocol, there was no gross examination of the nasal cavities. According to the sponsor the only potential treatment related histopathology was the erosion in nasal cavity of a high dose treated female and the epithelial hyperplasia in the maxiloturbinates in an additional high dose treated female. The sponsor notes that the apparent treatment related increase in incidence of fibrosing alveolitis in treated females (1/4-control, 2/4-low dose, 4/4 high-dose) was actually not treatment related, since it was considered "as a

resolving stage of pre-existing inflammation." It is not clear to the reviewer how this apparent treatment related effect can be dismissed in the context of a 13 week study. If the dogs had pre-existing (prior to the study initiation) fibrosing alveolitis which was resolving during the course of the study, then it is possible that treatment with is somehow inhibiting or slowing down the resolution. Also possibly noteworthy is the occurrence of pleural fibrosis/adhesion in a single high dose treated male and bronchiolitis associated with low grade epithelial hyperplasia in a single high dose treated male.

Again this study is problematic because its small size (4/sex/group) and is the only full study carried out with the sweetened formulation and as such, must stand alone. Because of this, it is difficult to dismiss findings seen only in single animals. Again, from the information provided in the original submission and the 1/28/96 amendment , it is not clear whether local irritation to the nasal cavity and the respiratory organs was adequately assessed in this study. Additionally, the stability studies on the test formulation indicated that the pH of the test formulation decreased by a unit over the course of the study.

Studies Using Preserved Formulation:

At one point in the product development the sponsor chose to pursue a preserved formulation, employing benzalkonium chloride (0.2% by volume) and phenylethyl alcohol (4% by weight). Four studies were carried out with this formulation, a pilot 8 day inhalation study in rats (#R12285), a 35 day inhalation study in rats (R12282), a pilot study to determine the maximum repeatable daily intranasal dosage level in dogs (#D12278) and a one month intranasal toxicity study in dogs (#D12279).

Study # D12279, the one month intranasal toxicity study in dogs, was not reviewed for this NDA. It was a GLP study with the following noteworthy exceptions: the final report was not audited by the quality assurance unit and the stability of the test article/carrier mixture was not determined over the complete range of concentrations used in the study. In addition, there were no summary tables for macroscopic pathology or histopathology, so to assess (review) these parameters, it would be necessary to go through each animals necropsy report (individual animal data form). Given the time constraints for this NDA, this was not done.

Study # D12278, the pilot study to determine the maximum repeatable daily intranasal dosage level in dogs, was also not reviewed for this NDA, it was a small, preliminary dose ranging study carried out according to GLP guidelines with many notable exceptions.

Study # R12282, the 35 day inhalation study in AHA rats and Study # R12285, the 8 day pilot inhalation study in rats were reviewed for this NDA, because they were the only studies conducted in rodents. The studies were carried out to examine the local irritation of a variant of the clinical formulation to the nasal cavity and respiratory organs. For both of these studies it is important to note that they are inhalation studies, which involved snout only exposure of the rat to aerosolized test formulation. In the study rationale the sponsor states that inhalation was chosen because intranasal administration to the rat is not practicable. It has been the Divisions's experience that it is possible to carry out intranasal dosing in rats.

Study # R12282, the 35 day inhalation toxicity study in rats, was a full toxicity study, rather than simply an irritancy study (see review page 44 -52). All of the parameters usually measured in a full toxicity study were measured. The test formulation used in this study was l at a concentration of 200 mg(base)/ml in the preservative containing vehicle. Dose was varied among treatment groups by varying time of exposure to the aerosolized drug. This study consisted of five treatment groups of 10/sex/group which were sacrificed after 35 days of treatment and an additional 5/sex from the air control group, the vehicle control group and the high dose treated group, which were retained, without additional treatment, for a two week recovery period. The air control group was given daily 60 minute exposures to air through the dosing apparatus. The vehicle control group was given daily 60 minute exposures to vehicle through the dosing apparatus. The low dose group (11.5 mg(base)/kg/day) was exposed daily to 15 minutes of the test formulation and 45 minutes of air through the test apparatus. The mid dose treated group (23.3 mg(base)/kg/day) was exposed daily to 30 minutes of the test formulation and 30 minutes of air through the test apparatus. The high dose group (45.5 mg(base)/kg/day) was treated daily with 60 minute exposure to the test formulation through the dosing apparatus.

In this study treatment related clinical signs included hair loss, staining of the head and snout and matted

fur. Macroscopic pathology revealed related alopecia, which persisted in females through the recovery period. There were also increased incidences of pale subpleural foci in the lungs from the vehicle control and the mid and high dose treated groups, which persisted in the high dose group through the recovery period.

Full histopathology was carried out on the air and vehicle control groups and the high dose treatment group. In addition, the nasal passages, larynx, trachea (including bifurcations) and lungs were examined for the low and mid dose treated groups. Treatment related histopathology was confined to the larynx and the tracheal bifurcation. With reference to the larynx, necrosis of the ventral cartilage was noted in all groups exposed to vehicle or vehicle plus drug. This problem occurred in almost all of the vehicle control, mid and high dose treated animals. The lower incidence in the low dose group may have been a reflection of the comparatively shorter exposure time to vehicle. Hyperplasia and keratinization of the laryngeal ventral epithelium occurred in the vehicle and treated groups; however, the incidence and severity increased with increasing dose. Ulceration of the ventral epithelium was noted in a single high dose female. There was a dose related increase in the incidence and severity of hyperplasia and hyperplasia associated with squamous metaplasia of the ventrolateral epithelium in treated animals, which did not occur in the vehicle control group. There were also a treatment related increases in incidence and severity of hyperplasia (mid and high dose groups) and keratinization (high dose group) of the lateral epithelium, which was not present in any control or low dose treated animals. There were also treatment related increases in the incidence and severity of hyperplasia and keratinization of the epithelium of the arytenoid projection in all treated groups. With reference to the tracheal bifurcation, there was an increased incidence of apparent loss of cilia from the epithelium in the high dose group.

Recall that only five animals per sex from the air and vehicle control groups and the high dose treatment group were maintained for a two week recovery period and only the larynx and tracheal bifurcations for each animal were examined. After the recovery period necrosis of the ventral cartilage was noted in 8 of 10 vehicle control rats and 10 of 10 high dose treated rats. There was still evidence of increased incidence of hyperplasia of the ventrolateral epithelium (larynx) in the high dose treated group and a treatment related increase in the severity of the hyperplasia of the epithelium of the arytenoid projections (larynx). There was also an apparent treatment related increase in subepithelial mononuclear cells in the arytenoid projection (larynx). The results of this aspect of the study suggest that the keratinization and the squamous metaplasia were reversible, as well as most of the hyperplasia.

Study # R12285, the 8 day inhalation toxicity study in rats, was a non-GLP pilot study for the 35 day toxicity study (see review pages 39 - 43). All of the parameters usually measured in a full toxicity study were measured. The test formulation used in this study was at a concentration of 200 mg(base)/ml in the preservative containing vehicle. Dose was varied among treatment groups by varying time of exposure to the aerosolized treatment formulation. This study consisted of four treatment groups of 5/sex/group which were sacrificed after 8-9 consecutive days of treatment. The air control group was given daily two hour exposures to air through the dosing apparatus. The vehicle control group was given daily two hour exposures to vehicle through the dosing apparatus. The low dose group (45.5 - 52.7 mg(base)/kg/day) was exposed daily to one hour of the test formulation through the test apparatus. The high dose group (91.5 - 111.3 mg(base)/kg/day) was treated daily with two hours of exposure to the test formulation through the dosing apparatus. In this study one high dose treated animal was sacrificed on day 4 for humane reasons. By day 2 this animal demonstrated hunched, low posture, labored breathing, gasping, pale extremities, half-closed eyes, red nasal staining and was cold to the touch. On day 3 it demonstrated labored, noisy breathing.

In general histopathology was carried out on the trachea, liver, larynx, lungs, kidneys, head (for nasal chambers), heart, femur (& bone marrow), and the mediastinal lymph node. The sponsor did not provide a summary histopathology table as part of this non-GLP study. In this study histopathological exam of tissues from the air control group was not conducted. Therefore, all comparisons were based on animals exposed to the vehicle with the preservative. Of note is the extent of apparent treatment related damage in the high dose treated male sacrificed after 3 days of drug exposure (alveolar hemorrhage, foamy macrophage aggregate in the lung, widespread submucosal acute inflammation of the larynx, epithelial hyperplasia and ulceration of the larynx, and squamous metaplasia of the lining of the ventral meatus of the nasal cavity.

These signs, plus epithelial hyperplasia on the arytenoid process and squamous metaplasia of the larynx represent the treatment related pathology. The sponsor states that these alterations were signs of irritancy in the upper respiratory tract.

These two inhalation studies in rats are problematic, since they also stand in isolation. They are the only studies reviewed using the preserved formulation. They are the only studies in rodents and the only studies by the inhalation route. Again, from the information provided in this study, it is not clear whether local irritation to the nasal cavity and the respiratory organs was adequately assessed in these studies.

The sponsor concluded based on the treatment related histopathology that the clinical use of this vehicle was not recommended.

Studies Using the Gel Formulation:

The sponsor submitted reports on three intranasal irritancy studies carried out employing the succinate salt of in a gel matrix. Due to time constraints these studies were not reviewed. These studies were: One week repeatable intranasal irritancy study of in beagle dogs (study # 110973), Second one week repeatable intranasal irritancy study of SN-308 ( in beagle dogs (study # 110974),Third one week repeatable intranasal irritancy study of SN-308 in beagle dog (study # 110975).

Miscellaneous Studies:

The sponsor supplied two non-GLP studies (# G11179, review pages 53 - 55 and # G11191, review pages 56 - 58), which looked at the potential o as a 10% or 50% w/v solution in water to cause skin irritation in the guinea pig skin from a single occluded applications to abraded and intact skin, 24 and 48 hours after application. According to the scoring system used in these studies, application of 10% w/v produced negligible irritancy to both the intact and abraded skin and no significant increase in skin-fold thickness at both 24 and 48 hours post dose. Application of 50% w/v GR43175N produced moderate irritation to the abraded skin and negligible irritation to the intact skin and resulted in a significant increase in skin-fold thickness in the abraded skin at both 24 hours and 48 hours post dose.

Table 1: Histopathology Summary from Intranasal Studies

| species | study # | formulation | concentration mg(base)/ml | dosing regimen | daily total dose mg(base) | effects | |
|---|---|---|---|---|---|---|---|
| dog 14 day | D13901 | buffered (clinical) | 200 | 0.3 ml, b.i.d. | 120 | 1/8 | focal squamous metaplasia of bronchial epithelium (grade 1) |
| dog 13 week | D12787 | unbuffered | 0 | 0.6 ml, b.i.d. | 0 | 1/8 | fibrosing alveolitis (slight focal) |
| | | | 200 | | 240 | 1/8 | fibrosing alveolitis (minimal) |
| | | | | | | 2/8 | bronchitis (slight - minimal) |
| | | | 400 | | 480 | 3/8 | fibrosing alveolitis (slight - minimal) |
| | | | | | | 2/8 | bronchitis (slight - moderately severe) |
| | | | | | | 2/8 | granuloma |
| | | | | | | 1/8 | lymphoid hyperplasia-larynx (minimal) |
| | | | | | | 1/8 | lymphoid hyperplasia of respiratory region of nasal passages (minimal) |
| dog 13 week | D13342 | sweetened | 0 | 0.6 ml, b.i.d. | 0 | 1/8 | fibrosing alveolitis (minimal) |
| | | | | | | 1/8 | granuloma (lung) |
| | | | | | | 1/8 | reactive bronchial lymph node (minimal) |
| | | | 200 | | 240 | 2/8 | fibrosing alveolitis (minimal) |
| | | | | | | 3/8 | granuloma (lung) (*) |
| | | | 400 | | 480 | 4/8 | fibrosing alveolitis (minimal) |
| | | | | | | 1/8 | pleural fibrosis/adhesion (moderate - multifocal) |
| | | | | | | 4/8 | granuloma (lung) (*) |
| | | | | | | 1/8 | bronchiolitis associated with low grade epithelial hyperplasia |
| | | | | | | 3/8 | reactive bronchial lymph node (minimal) |
| | | | | | | 1/8 | focal erosion of nasal cavity (minimal) |
| | | | | | | 1/8 | epithelial hyperplasia (maxilloturbinates) (minimal) |
| monkey 14 day | P11224 | unbuffered | 200 | 0.2 ml, q.i.d. | 160 | - | |
| | | | 400 | | 320 | 1/4 | focal pulmonary fibrosis and pleural adhesion |

(*) dose related increase in degree affected

| Table 2a: Histopathology Results from Preliminary Study in Rats | | |
|---|---|---|
| | | 3 days of treatment |
| hours of exposure to test formulation (or vehicle) | | 2 |
| lung | foamy macrophage aggregate | 1/1 |
| lung | alveolar hemorrhage | 1/1 |
| larynx | widespread submucosal acute inflammation | 1/1 |
| larynx | epithelial hyperplasia of ventral region | 1/1 |
| larynx | ulceration in ventral region | 1/1 |
| nasal cavity | squamous metaplasia lining of the ventral meatus | 1/1 |

| Table 2b: Histopathology Results from Preliminary Study in Rats | | | | |
|---|---|---|---|---|
| | | 8 days of treatment | | |
| | | vehicle | low dose | high dose |
| hours of exposure to test formulation (or vehicle) | | 2 | 1 | 2 |
| lung | foamy macrophage aggregate | 0/10 | 1/10 | 1/9 |
| larynx | submucosal inflammatory cell infiltrate | 1/10 | 0/10 | 3/8 |
| larynx | epithelial hyperplasia on arytenoid process | 0/10 | 9/10 | 6/8 |
| larynx | epithelial hyperplasia (ventral region) | 0/10 | 1/10 | 7/8 |
| larynx | squamous metaplasia | 0/10 | 2/10 | 6/8 |
| larynx | mononuclear cell focus in ventral pouch | 0/10 | 0/10 | 1/8 |
| larynx | small glandular cyst(s) ventral pouch | 0/10 | 2/10 | 0/8 |

| Table 3: Histopathology Results from Main Study in Rats | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 35 days of treatment | | | | | 35 days of treatment + 2 week recovery period | | |
| | | air control | vehicle control | low dose | mid dose | high dose | air control | vehicle control | high dose |
| hours exposure to test formulation (or vehicle or air) | | 1 | 1 | 0.25 | 0.5 | 1 | 1 | 1 | 1 |
| larynx | necrosis of the ventral cartilage | 0/20 | 17/20 | 9/20 | 18/20 | 20/20 | 0/10 | 8/10 | 10/10 |
| larynx | ventral epithelium<br>- hyperplasia<br>- keratinization | 0/20<br>0/20 | 7/20<br>2/20 | 10/20<br>0/20 | 15/20<br>2/20 | 17/20<br>8/20 | 0/10<br>0/10 | 2/10<br>0/10 | 0/10<br>0/10 |
| larynx | ventrolateral epithelium<br>- hyperplasia<br>- hyperplasia with squamous metaplasia | 0/20<br>0/20 | 0/20<br>0/20 | 2/20<br>2/20 | 5/20<br>2/20 | 0/20<br>14/20 | 1/10<br>0/10 | 1/10<br>0/10 | 3/10<br>0/10 |
| larynx | lateral epithelium<br>- hyperplasia<br>- keratinization | 0/20<br>0/20 | 0/20<br>0/20 | 0/20<br>0/20 | 7/20<br>0/20 | 12/20<br>7/20 | 0/10<br>0/10 | 0/10<br>0/10 | 0/10<br>0/10 |
| larynx | epithelium of the arytenoid projection<br>- hyperplasia<br>- keratinization | 9/20<br>1/20 | 7/20<br>0/20 | 18/20<br>2/20 | 16/20<br>10/20 | 18/20<br>13/20 | 5/10<br>0/10 | 5/10<br>0/10 | 5/10<br>0/10 |
| tracheal bifurcation | epithelium<br>- apparent loss of cilia | 1/20 | 0/20 | 1/20 | 0/20 | 3/20 | 1/10 | 1/10 | 1/10 |

**Outstanding Issues**

Primary Outstanding Issues:

1. Adequacy of sampling in the studies reviewed:

   a. From the information provided in the original submission and the 1/28/96 amendment, it is not clear whether local irritation to the nasal cavity and the respiratory organs was adequately assessed in each study. Generally in the nasal cavity and parts of the respiratory system, the highly specific regional histopathology changes dramatically over small distances. Descriptions of the positions of the cuts, which determine the tissue blocks for histopathology, in isolation do not provide assurance that histopathological examinations of the nasal cavity and respiratory organs included all of the appropriate representative types of epithelium and tissue (e.g., the squamous, the respiratory and the olfactory epithelium of the nasal cavity and transition zones).

   b. Study # 13901, the 14 day intranasal irritancy study in dogs carried out with the buffered formulation, is the pivotal intranasal toxicity study for this NDA. It is the only intranasal irritancy study carried out with the proposed clinical formulation. In this study there was a change in protocol after the administration of the first dose, such that from day 2 onward only the right nostril was treated for each animal, instead of both nostrils. Therefore, for this pivotal study, toxicity to the nasal cavity was based on the evaluation of eight drug treated nostrils.

2. Interpretation of apparent treatment related histopathologic findings:

   a. Isolated findings in small studies in dogs and monkeys cannot easily be dismissed (see summary Table 1, page 66). These studies, to a certain degree, stand in isolation. For dogs, each study was carried out with different formulations. These changes in the test formulations could potentially alter the irritation profile for each study. Only one very small intranasal study was conducted in monkeys. There are no other studies in monkeys with which to compare the results.

   In the two 13 week intranasal studies in dogs there were apparent treatment related increased incidences of fibrosing alveolitis (minimal) and in the incidence of granulomas in the lungs. Other findings in the dog studies were generally isolated incidences of squamous metaplasia, lymphoid hyperplasia or epithelial hyperplasia of the nasal cavity or respiratory organs. There was a single incidence of pulmonary fibrosis and pleural adhesion in the high dose treated monkeys.

   b. For the rat, there were two inhalation studies, an 8 day and a 35 day study with the preserved formulation. In the 35 day study, the major site of treatment related toxicity was the larynx. In: Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation by P. Greaves (Elsevier, 1990, pages 192-193), the author states: "The larynx of rodents is particularly susceptible to the effects of inhaled substances.....Lesions tend to occur in the ventrolateral region which is covered by respiratory epithelium and the inner aspect of the arytenoid processes which is lined by squamous mucosa."

   Interpretation of the results of the 35 day inhalation study in rats (see summary Table 3, page 67) is complicated by the toxicity of the vehicle. The vehicle caused necrosis of the ventral cartilage of the larynx and some incidence of hyperplasia and keratinization of the ventral epithelia of the larynx and hyperplasia of the epithelia of the arytenoid projection of the larynx. The addition resulted in increased incidence and severity of these findings and, in addition, resulted in hyperplasia and hyperplasia with squamous metaplasia of the ventrolateral epithelium of the larynx, hyperplasia and keratinization of the lateral epithelium of the larynx, and keratinization of the epithelium of the arytenoid projection of the larynx, as well as a slight increase in incidence of apparent loss of cilia from the tracheal bifurcation. This study incorporated a two week recovery period for the controls and high dose groups. The results of this aspect of the study suggest that the

hyperplasia. After the recovery period only the hyperplasia of the vetrolateral epithelium appears to be a treatment related response.

It should be noted that in the 8 day pilot inhalation study in rats (see summary Table 2a and 2b, page 67), which exposed the vehicle group and the high dose treated group for longer periods of time daily than the 35 day study, there was no evidence of toxicity in the vehicle group. After eight days of treatment in both the high and low dose group (1 hour and 2 hour daily exposure respectively ) there was evidence of treatment related hyperplasia and squamous metaplasia of laryngeal epithelium. In one high dose animal sacrificed after 3 days of treatment there was already evidence of epithelial hyperplasia and erosion in the larynx and squamous metaplasia of the lining of the ventral meatus of the nasal cavity (which did not occur in the 35 day study).

3. In study # D12787, the 13 week intranasal tolerance study in dogs employing the unbuffered formulation, there was a notation of superficial corneal opacities in one low dose treated dog and two high dose treated dogs on day 6. This finding resulted in a change in the protocol such that for all dogs the eyes were covered during dosing. In study # D13342, the 13 week intranasal irritancy study in dogs employing the sweetened formulation, the sponsor notes the occurrence of ocular opacity in a high dose treated dog during week 1. These findings were not discussed further, and there is no indication of follow-up examination of the eyes.

   In study # D12279, a one month intranasal toxicity study employing the preserved formulation, and not formally reviewed for this NDA, there were notations of corneal stippling or a pitted appearance at all doses tested and ranging in incidence from occasional to frequent. There was no further discussion of this sign; however, according to the protocol ophthalmoscopic examination took place prior to the initiation of the study and on study day 30. Although no data was supplied for this aspect of the study the sponsor states that there were no changes due to treatment.

   These finding may be similar or related to the corneal opacity seen with oral or subcutaneous dosing of dogs with sumatriptan succinate (see Addendum to Pharmacologist's Review for NDA 20-132, dated 1/13/95), or it may be an independent finding, possibly associated with accidental exposure of the eye to the test formulation spray. Histopathologic examination of the eyes was not routinely carried out.

4. With reference to study # P11224, the 14 day intranasal irritancy study in monkeys employing the unbuffered formulation, there were two related issues concerning the stability of the test formulation, which could alter the acceptability of the study. The study report states that the day 14 samples of the test formulation were returned to the sponsor for analysis. The data presented for this analysis was for solutions of nominal concentrations of 5, 10 , 20 , 40 , 80 and 100 mg(base)/ml. The formulations used in this study were 200 and 400 mg(base)/ml. In addition, according to the study protocol, each day fresh solutions of test substance were added to the residue in the nasal spray bottle from the previous days's dosing. This could potentially lead to a problem of a less potent, more contaminated test solutions being used as the study progressed.

Other Outstanding Issues:

5. The sponsor has provided a variation of the Draize test to assess ocular irritancy of the clinical formulation. This study may not have been adequate by design to provide a definitive evaluation. In addition, this study includes a GLP statement based on UK regulations, with the limitation that this study may not have been subjected to a procedural inspection by the QA unit. Does the review Division have any formal requirements for ocular irritation studies?

6. Study # D12279, the one month intranasal toxicity study in dogs using the preserved formulation, was not reviewed for this NDA. There were no summary tables for macroscopic pathology or histopathology. In order to review these parameters, it would be necessary to go through each animals necropsy report (individual animal data form). The sponsor should put together appropriate summary tables and submit them to this NDA for review.

7. Three intranasal irritancy studies, of one week durations, in beagle dogs employing the succinate salt c [redacted] in a gel matrix, were submitted to the NDA. Due to time constraints these studies

**Conclusions**

The sum total of the preclinical data submitted to this NDA does not support approval of Imitrex by the nasal route. Treatments for migraine have traditionally been considered chronic-intermittent use drugs, following the preclinical requirements for chronic use drugs. Imitrex (sumatriptan, has been studied extensively in dogs and rats by the oral and subcutaneous routes (see Pharmacology/Toxicology reviews for NDA 20-080 and NDA 20-132). These studies could be used to support the systemic toxicity of intranasally administered sumatriptan, provided that the sponsor has shown that the plasma metabolic profiles for sumatriptan are similar among the different routes of administration, and that the metabolic profiles from animals treated by the nasal route are similar to that produced by the humans treated by the nasal route. The sponsor has not studied metabolism in humans after intranasal administration of sumatriptan. The sponsor has provided urinary metabolic profiles in rats and dogs after intranasal and intravenous administration.

The Division of Pulmonary Drug Products has an extensive history of reviewing drugs administered by the intranasal route. That Division generally requires preclinical toxicity studies in two species (rodent and non-rodent) of appropriate duration to support the proposed clinical use. The requirements for chronic use drugs currently consist of a six month study in rodents and 12 month study in non-rodents by appropriate routes. That Division is willing to make exceptions, on a case by case basis, for drugs which are to be administered intranasally, and which are fully characterized by the oral route in two species. In certain cases that Division is willing to accept a single six month intranasal or inhalation study in an appropriate species as the chronic toxicity study. The sponsor must make the case for which species is the most appropriate.

With reference to the issue of carcinogenic potential of a drug administered by the intranasal route, the Division of Pulmonary Drug Products will accept intranasal studies, or inhalation studies, if the sponsor can demonstrate that during the inhalation studies the nasal tissue is exposed to a adequate levels of drug. In that Division, there are certain circumstances under which oral carcinogenicity studies can be used to support an intranasal drug. In order to meet the criteria, the chronic intranasal toxicity studies must not have demonstrated histopathology which is suggestive of proliferative or pre-neoplastic changes and the sponsor must have carried out the necessary pharmacokinetic studies to demonstrate adequate local (i.e., nasal and respiratory tissue) exposure to the drug by the oral route.

In general, the reviewer agrees with the basic requirements and suggestions from the Division of Pulmonary Drug Products. For this NDA no chronic intranasal toxicity studies have been submitted. For sumatriptan, only one intranasal irritancy study with the clinical formulation has been submitted. This was a 14 day intranasal irritancy study in dogs. The sponsor also supplied the report of a small study in rabbits carried out with the clinical formulation to determine the potential for ocular irritancy.

The sponsor has provided several other intranasal irritancy and/or intranasal toxicity studies in dogs using test formulations which were not equivalent to the clinical formulation, which is buffered. These studies employed, unbuffered solutions, unbuffered - sweetened (saccharin) solutions, unbuffered - preserved solutions (not reviewed), and a nasal gel formulation (also not reviewed). The studies employing the unbuffered and unbuffered-sweetened solutions were 13 weeks in duration. The sponsor has provided a very small 14 day intranasal irritancy study in monkeys employing the unbufferd formulation. The sponsor also carried out two inhalation toxicity studies in rats, a pilot 8 day study and 35 day study, using the unbuffered-preserved formulation.

The longest duration study for dogs was 13 weeks, for rats was 35 days and for monkeys was 14 days. For the intranasal studies in dogs and monkeys, animals were treated with the test formulations two to four times per day. For the inhalation studies in rats, animals were exposed (snout only) to the test formulations daily for periods of 15 minutes to two hours. None of these employed the clinical formulation. None of these studies alone, or considered in total, is of sufficient duration to support chronic use in humans. Each one of the studies reviewed, except the two week intranasal study in monkeys, demonstrated some signal of treatment related hyperplasia or metaplasia of the nasal cavity or respiratory organs. This warrants a more thorough preclinical examination of sumatriptan by the intranasal route prior to marketing, especially since histopathology is not available from humans and that this drug is already marketed for migraine by two other routes (oral and subcutaneous).

**Recommendations**

The preclinical toxicity studies submitted do not support approval.

1. The sponsor must supply plasma metabolite profiles after intranasal administration in humans, rat and dogs. There should also be comparable profiles in the same species by the oral and/or subcutaneous routes. The sponsor should then compare the plasma metabolic profile among species and between routes.

2. The sponsor, at a minimum, must carry out a six month intranasal irritancy study in the most appropriate species with the clinical formulation. The sponsor must provide a strong rationale for their choice of species. If the chronic intranasal toxicity study demonstrates findings suggestive of treatment related proliferative or pre-neoplastic changes, the sponsor may have to carry out carcinogenicity studies by the intranasal route.

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Andrea M. Powell

Andrea M. Powell, Ph.D.

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Original NDA 20-626
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HFD-120/GFitzgerald ggf 7/1/96
HFD-120/APowell
HFD-502/JDeGeorge

Revisions to original review completed 4/19/96

## ADDENDUM TO PHARMACOLOGY REVIEW FOR NDA 20-626

Reviewer: Andrea M. Powell, Ph.D.
Date: 5/13/96

This addendum reflects the content of a portion of a telephone conversation between the reviewer and Dr. Scott L. Eustis, D.V.M., Ph.D. from National Institute of Environmental Health Sciences (Research Triangle Park, NC), which took place in September of 1993. This telephone conversation was initiated by the reviewer to discuss nasal pathology issues from IND reports for intranasal migraine drugs.

Based on Dr. Eustis' comments, it may be helpful for the interpretation of the data presented in study # R12282, the 35 day inhalation toxicology study in rats, to request from the sponsor a further explanation of the histopathology terms (i.e., type of epithelium (squamous, respiratory, etc.) and further explanation of the keratinization) reported for the larynx, as detailed below:

1. hyperplasia and keratinization of the laryngeal ventral epithelium
2. hyperplasia and hyperplasia associated with squamous metaplasia of the ventrolateral epithelium
3. hyperplasia and keratinization of the lateral epithelium
4. hyperplasia and keratinization of the epithelium of the arytenoid projection

Similar information should be supplied for study # R12285, the 8 day inhalation toxicity study in rats.

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## ADDENDUM TO PHARMACOLOGY REVIEW FOR NDA 20-626

Reviewer: Andrea M. Powell, Ph.D.
Date: 5/15/96

As part of this NDA, the sponsor submitted study # R20569 "Lack of activity in a micronucleus test following oral administration to male and female PVG rats" (Glaxo report # WPT/94/333, volume 15). This study supplements the micronucleus test in PVG rats submitted to the original NDA for sumatriptan injection (NDA 20-080). In that study male PVG rats (5/group) were treated with single oral doses of [redacted] at dose levels of 100, 300 and 1000 mg(base)/kg and bone marrow was sampled at 24 and 48 hours post dose as summarized in the following reviewer generated table. In that assay there were no statistically significant increases in the number of micronuclei in the [redacted] treated group over the vehicle control indicating chromosome damage and there was no clear dose-related change in erythrocyte proliferation.

| # micronucleated immature erythrocytes per 1000 immature (polychromatic) erythrocytes | | | | |
|---|---|---|---|---|
| group | 24 hr sacrifice # M-PCE/PCE counted | 24 hr sacrifice % micronuc. PCEs | 48 hr sacrifice # M-PCE/PCE counted | 48 hr sacrifice % micronucl. PCEs |
| negative control | 2/5000 | 0.04% | 2/3000 | 0.07% |
| positive control | 156/5000 | 3% | 203/5000 | 4% |
| 100 mg(base)/kg | 4/5000 | 0.08% | 3/4000 | 0.08% |
| 300 mg(base)/kg | 3/5000 | 0.06% | 2/5000 | 0.04% |
| 1000 mg(base)/kg | 5/5000 | 0.1% | 5/5000 | 0.1% |
| The smears from three animals (two negative control, 48 hr sacrifice and one [redacted] (100 mg(base)/kg) treated, 48 hr sacrifice) were not evaluated because of poor quality of the smear. | | | | |

The current study was carried out at Glaxo Research and Development Ltd. in the UK according to GLP principles between 8/2/94 and 8/4/94. [redacted] (batch # E93L2572 - Montrose Batch 4007) was dissolved in sterile water for irrigation, which also served as the vehicle control. [redacted] dissolved in sterile water, served as the positive control. PVG rats (5/sex/group) were administered a single oral (gavage) dose of 20 mL/kg of the appropriate test solution (GR43175C - 2 g(base)/kg or cyclophosphamide - 20 mg/kg). The animals were sacrificed at either 24 or 48 hours post dose and bone marrow samples were obtained. The results are summarized in the following reviewer-generated tables.

| Individual and Mean Micronucleated Polychromatic Erythrocyte (MPCE) Counts | | | | | |
|---|---|---|---|---|---|
| treatment | dose (mg(base)/kg) | time (hrs post dose) | sex | MPCE per 2000 PCE | |
| | | | | individual values | mean |
| vehicle control | 0 | 24 | male<br>female | 2, 4, 3, 1, 4<br>3, 2, 3, 3, 5 | 2.8<br>3.2 |
| | 2000 | 24 | male<br>female | 2, 2, 6, 1, 4<br>1, 3, 5, 2, 2 | 3.0<br>2.6 |
| cyclophosphamide | 20 | 24 | male<br>female | 33, 36, 25, 21, 23<br>21, 23, 34, 28, 24 | 27.6<br>26.0 |
| vehicle control | 0 | 48 | male<br>female | 4, 1, 2, 0, 2<br>1, 2, 3, 4, 2 | 1.8<br>2.4 |
| | 2000 | 48 | male<br>female | 4, 3, 4, 2, 2<br>2, 3, 3, 2, 3 | 3.0<br>2.6 |

| Individual and Mean Polychromatic Erythrocyte (PCE) Proportion per Total Erythrocyte Population of 1000 Cells | | | | | |
|---|---|---|---|---|---|
| treatment | dose (mg(base)/kg) | time (hrs post dose) | sex | proportion of PCE per 1000 cells | |
| | | | | individual values | mean |
| vehicle control | 0 | 24 | male<br>female | 0.36, 0.42, 0.40, 0.35, 0.39<br>0.48, 0.41, 0.38, 0.49, 0.38 | 0.38<br>0.43 |
| | 2000 | 24 | male<br>female | 0.31, 0.34, 0.38, 0.34, 0.37<br>0.38, 0.39, 0.38, 0.41, 0.50 | 0.35<br>0.41 |
| cyclophosphamide | 20 | 24 | male<br>female | 0.35, 0.39, 0.25, 0.34, 0.32<br>0.37, 0.32, 0.37, 0.36. 0.36 | 0.33<br>0.36 |
| vehicle control | 0 | 48 | male<br>female | 0.45, 0.34, 0.33, 0.39, 0.33<br>0.37, 0.40, 0.44, 0.41, 0.42 | 0.37<br>0.41 |
| | 2000 | 48 | male<br>female | 0.41, 0.41, 0.39, 0.35, 0.52<br>0.39, 0.34, 0.40, 0.45, 0.42 | 0.42<br>0.40 |

In this assay [redacted] did not induce an increase in the number of micronuclei, over the concurrent vehicle control, nor did it cause a marked reduction in the frequency of polychromatic erythrocytes.

The results of this study do not affect the current labeling of sumatriptan.

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