CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-359

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	N 021359
Supporting document/s:	64
Applicant's letter date:	December 20, 2010
CDER stamp date:	December 21, 2010
Product:	Nitroglycerin ointment 0.4%
Indication:	Treatment of moderate to severe pain associated with chronic anal fissure
Applicant:	Prostrakan Inc.
Review Division:	DAAAP
Reviewer:	Newton H. Woo, Ph.D.
Supervisor/Team Leader:	Adam Wasserman, Ph.D.
Division Director:	Bob Rappaport, M.D.
Project Manager:	Christopher Hilfiger

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction and Regulatory Background

The present application by Prostrakan is a resubmission of NDA 21-359 for a nitroglycerin ointment 0.4%[1], formerly referred to as Rectogesic and Cellegesic, developed for the indication of chronic anal fissure pain. The regulatory history of NDA 21-359 is summarized in the Table below.

Submission Date	Review Division	Action Date	Agency Action
2001	DCRP	2002	Withdrawn prior to decision (nonclinical review submitted)
2004	DCRP	2004	Not Approved
2005	DCRP	2006	Approvable
2009	DAARP	2010	Complete Response
2010	DAAAP	2011	Pending (Current cycle)

The initial cycle of NDA 21-359, was reviewed by the Division of Cardio-Renal Drug Products (DCRP) in 2002. In the original NDA submission, published literature was submitted as nonclinical support for marketing approval, which was reviewed by Dr. A.G. Proakis (nonclinical review dated April 10, 2002). There were no nonclinical issues identified and a recommendation of "may be approved" was documented; however, the NDA was withdrawn prior to Agency Action. Resubmitted in 2004, marketing approval of NDA 21-359 was denied by DCRP due to an inadequate demonstration of clinical efficacy and problematic statistical analyses. In 2005, the NDA was amended and resubmitted and was deemed Approvable by DCRP. However approval was on the condition that an additional clinical study was conducted and the results of which provided substantial evidence that the drug was effective.

In 2009 the NDA was resubmitted to the Division of Anesthesia, Analgesia and Rheumatology Products. Upon re-review of the NDA as part of the 2009 cycle, outstanding clinical issues along with a newly identified nonclinical deficiency led to the recommendation of "should not be approved", which resulted in a Complete Response issued to the Sponsor on March 30, 2010. The nonclinical deficiency stemmed from the lack of support for establishing that systemic and metabolite levels with use of the nitroglycerin ointment 0.4% fall within levels of the Referenced Drug Nitro-Dur (NDA 20-145). Consequently additional nonclinical studies were required.

The discrepancy between the nonclinical review in 2002 and 2010 between the two Divisions arises from the confusion of the Listed Product and the changing regulatory understanding of 505(b)(2) requirements. In the original submission of 2001, the original 505(b)(2) application did not identify a Listed Drug Product as this section on Form 356h was blank. However, in the cover letter, the Applicant discussed the levels of

[1] The Trade Name has not yet been determined

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nitroglycerin in an approved ointment (i.e. Nitrol®; NDA 18-774)[2] and as a result the original nonclinical review was likely based on the prior determination of safety of Nitrol. When the NDA was resubmitted in 2004, the recently changed requirements for a b(2) NDA application required the Applicant to identify a Reference drug, which was in this case Nitro-Dur (NDA 20-145). Because the nonclinical review was already completed for the first cycle of the withdrawn NDA, this nonclinical review was used in the final second cycle Divisional memo for the resubmitted NDA and was not revisited for the third cycle. Therefore there was no mention of bridging or the requirement of additional nonclinical information. However, when NDA 21-359 was resubmitted to DAARP in 2009, nonclinical review recommended the application not be approved and identified as a deficiency the lack of evidence that levels of nitroglycerin and its metabolites after maximal indicated use of the nitroglycerin ointment 0.4% produces levels that are within that observed with the listed product, Nitro-Dur. Note the clinical dose of 0.4% is two-fold greater than what was used in the original pharmacokinetic study of 0.2% (Study NTG 98-02-02) that was submitted in the initial NDA. In the absence of appropriate clinical pharmacokinetic data for the nitroglycerin ointment 0.4%, resolution of the nonclinical deficiency required resolution through conduct of repeat-dose toxicology studies using the intra-rectal route in two species (one non-rodent) and nonclinical toxicokinetic bridging studies to establish safety margins for animal reproductive toxicology and carcinogenicity studies described in the label.

On June 17, 2010, a Type A meeting was held to discuss the 2010 Complete Response action, which included both clinical and the newly identified nonclinical deficiency. In the meeting package and a subsequent information amendment, the Applicant submitted literature support providing evidence that exposure levels of nitroglycerin and its metabolites with use of nitroglycerin ointment 4% fall within those reported with Nitro-Dur, which was deemed adequate by the Clinical Pharmacology and Pharmacology/Toxicology Staff and consequently the Applicant did not have to submit further nonclinical studies. With respect to the label, past nonclinical studies for animal reproductive toxicology and carcinogenicity studies were conducted with dietary nitroglycerin in which systemic and tissue exposure were not determined. As nitroglycerin undergoes extensive first-pass metabolism, the bioavailability for oral nitroglycerin is extremely low as compared to other routes of administration and therefore safety margins cannot be readily calculated. Consequently, safety margins are not reported in various labels of marketed nitroglycerin drug products, including the referenced drug Nitro-Dur. Carcinogenicity studies are not required for this drug product since the Medical Team does not expect the product to be used for six months in duration continuously or intermittently in a lifetime (For more detail see the clinical review by Neville A Gibbs, MD). Also, as this drug product is not considered to represent an increased risk relative to the listed drug, we will not require repeat of nonclinical studies to further clarify the label. Taken together, there are no outstanding pharmacology/toxicology issues with this NDA.

[2] The patent certification page was against patents held by an individual on the use of nitroglycerin for anal pain.

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1.2 Brief Discussion of Nonclinical Findings

The Applicant is relying on the Agency's prior finding of safety as well as the pharmacology, pharmacokinetics, and toxicology information described in the label of the listed drug Nitro-Dur. The Applicant bridged the proposed product to the listed product via literature support. A BA/BE study was not conducted, however the Applicant submitted information collated from the literature that demonstrated that nitroglycerin AUC and Cmax values from nitroglycerin ointment 4% were within that of Nitro-Dur® 40 cm^2 (0.8 mg NTG/hr) patch. It is noted that the submitted data had several underlying assumptions and extrapolations that were deemed acceptable by the reviewing Clinical Pharmacologist, Dr. David Lee. The product is considered to have adequate support for systemic safety based on this determination.

Nitroglycerin products have been approved for intravenous, sublingual and transdermal administration; however, this application, which is for rectal administration, is a new route of administration. Nonclinical studies in rabbits were conducted to support the safety of this route of administration but, as was noted in Dr. Lawrence Leshin's nonclinical review, the assessment for local toxicity was inadequately conducted. However, because clinical experience with nitroglycerin ointment 4% in several Phase III studies (REC-C-001, NTG 98-03-01, NTG 00-03-01, CP125 03-03-01) in which trial subjects received the ointment for at least 21 days up to 56 days demonstrated a lack of local adverse events, sufficient evidence for local safety is provided and additional local toxicology studies were not required.

Finally, the drug product formulation is considered acceptable.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical pharmacology and toxicology perspective, NDA 21-359 may be approved.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies are required at this time.

1.3.3 Labeling

The Table below contains the draft labeling submitted by the Applicant, the proposed changes and the rationale for the proposed changes. For the final version of the label, please refer to the Action Letter. The recommended changes from the proposed labeling are in strikeout or red font.

Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes

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(b) (4)	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** *Pregnancy Category C* Animal reproduction and teratogenicity studies have not been conducted with BRANDNAME. Nitroglycerin was not teratogenic when administered by topical or dietary route. There are no adequate and well-controlled studies in pregnant women. BRANDNAME should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Teratology studies in rats and rabbits were conducted with topically applied nitroglycerin ointment at doses up to 80 mg/kg/day and 240 mg/kg/day, respectively. No toxic effects on dams or fetuses were seen at any dose tested. A teratogenicity study was conducted in rats with nitroglycerin administered in the diet at levels up to 1% content (approximately 430 mg/kg/day) on days 6 to 15 of gestation. In offspring of the high-dose group, an increased but not statistically significant incidence of diaphragmatic hernias was noted together with decreased hyoid bone ossification. The latter finding probably reflects delayed development, thus indicating no clear evidence of a	**This section is largely identical to the Nitro-Dur label but has been adapted to conform to the Physician Labeling Rule and Structured Product Labeling format and in accordance with the CFR regarding the Pregnancy Category C.** **Note: The dietary teratogenicity study is not present in the Nitro-Dur label but it is the opinion of this reviewer that this newly added section should remain in the label as it provides additional relevant information. These results are taken from a non-proprietary published study in the public domain (Ellis et al., 1978) and appear in other nitroglycerin product labeling.**

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(b) (4)	potential teratogenic effect of nitroglycerin. **8.3 Nursing Mothers** It is not known whether nitroglycerin is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when BRANDNAME is administered to a nursing woman. **8.4 Pediatric Use** The safety and effectiveness of BRANDNAME in pediatric patients under 18 years of age have not been established.	
(b) (4)	**13 NONCLINICAL TOXICOLOGY** **Carcinogenesis, Mutagenesis, Impairment of Fertility** Animal carcinogenicity studies with topically applied nitroglycerin have not been performed. Rats receiving up to 434 mg/kg/day of dietary nitroglycerin for 2 years developed dose-related fibrotic and neoplastic changes in liver, including carcinomas, and interstitial cell tumors in testes. At the highest dose, the incidences of hepatocellular carcinomas was 52% compared to 0% in	**This section is identical to the Nitro-Dur label but has been adapted to conform to the Physician Labeling Rule and Structured Product Labeling format. Minor edits (in red) are suggested along with updated language for the genotoxicity section, which refers to results taken from non-proprietary literature (Ellis et al., 1978;**

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(b) (4)	untreated controls. Incidences of testicular tumors were 52% vs. 8% in controls. Lifetime dietary administration of up to 1058 mg/kg/day of nitroglycerin was not tumorigenic in mice. Nitroglycerin was negative in a rodent dominant lethal assay but was mutagenic in the *in vitro* bacterial reverse mutation (AMES) assay. A similar mutation in only one strain of *S. typhimurium* was also reported with other NO donors. There was no evidence of clastogenic potential in an *in vitro* Chinese Hamster Ovary assay that was conducted in the absence of metabolic activation or in several *in vivo* chromosomal aberration assays conducted in rats and dogs. In a three-generation reproduction study, rats received dietary nitroglycerin at doses up to about 434 mg/kg/day for 6 months prior to mating of the F_0 generation with treatment continuing through successive F_1 and F_2 generations. The high dose was associated with decreased feed intake and body weight gain in both sexes at all matings. No specific effect on the fertility of the F_0 generation was seen. Infertility noted in subsequent generations, however, was attributed to increased interstitial cell tissue and aspermatogenesis in the high-	**Maragos et al., 1993).** **Note: Nonclinical studies conducted with dietary nitroglycerin are problematic since nitroglycerin undergoes extensive first-pass metabolism and bioavailability for oral nitroglycerin is extremely low as compared to other routes of administration, including rectal administration. Therefore safety margins cannot be readily calculated and were not included in the present label. It is also noted that safety margins are not documented in other marketed nitroglycerin drug product labels.**

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(b) (4)	dose males.	**The last section "Animal Toxicology and/or Pharmacology" was deleted since it is not deemed clinically significant animal data necessary for safe and effective use of the drug in humans. It is also noted that this section does not appear in other nitroglycerin class labels.**

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

/s/

NEWTON H WOO
05/20/2011

ADAM M WASSERMAN
05/20/2011
I concur with Dr. Woo that the application may be approved from the nonclinical standpoint.

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

Supervisory Pharmacologist Memorandum

NDA NUMBER:	**21-359**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**Sept 30, 2009**
PRODUCT:	
(Proposed) Trade Name:	**Rectogesic**
Established Name:	**Nitroglycerin ointment 0.4%**
INTENDED CLINICAL POPULATION:	**Chronic Anal Fissure**
SPONSOR:	**ProStrakan International Ltd.**
REVIEW DIVISION:	**Division of Anesthesia and Analgesia Products**
PHARM/TOX REVIEWER:	**L. Steven Leshin, DVM, Ph.D.**
PHARM/TOX SUPERVISOR:	**Adam Wasserman, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**

I. BACKGROUND

The present submission is in response to a Not Approvable decision in December 2004 on a prior review cycle of NDA 21-359 for Cellegesic™ (nitroglycerin) 0.4% Ointment which at that time being was developed by Cellegy Pharmaceuticals. Marketing approval was denied due to an inadequate demonstration of efficacy. No nonclinical issues were identified and the original nonclinical review by the Division of Cardio-Renal Drug Products in 2002 recommended approval, principally based on the long history of clinical use of nitroglycerin-based products for angina, congestive heart failure, and myocardial infarction under 505(b)(2) pathway as well as published literature containing nonclinical studies which suggested nonclinical findings of concern (tumors in rat carcinogenicity studies, mutagenicity, male reproductive toxicity) were of unknown relevance given the duration of prior human experience with nitroglycerin-based pharmaceuticals. The original review also suggested the lower nitroglycerin concentrations (0.4% maximum) were well below the approved topical ointment concentration (2%) which the team believed likely would result in a lower systemic exposure. The present Applicant, ProStrakan Inc., has resubmitted with new clinical studies intended to address the deficiency noted in the NA letter of 2004 and proposed a new tradename, Rectogesic™. The current product, as Rectogesic, is licensed in the EU in 18 countries as well as Switzerland and has been marketed since 1995.

II. MAJOR NONCLINICAL ISSUES IDENTIFIED IN PRIMARY REVIEW

Dr. Leshin has noted that the nonclinical literature supporting nitroglycerin marketing is largely, though not exclusively, based on dietary administration which can be expected to produce a markedly reduced systemic exposure to nitroglycerin (GTN) and the active intermediate nitric oxide as presystemic hepatic biotransformation by glutathione-organic nitrate reductase to less active (but longer circulating) metabolites 1,3- and 1,2-glyceryl dinitrate (1,3- and 1,2-GDN, respectively) and subsequent mononitrate metabolites, glycerol and organic nitrate is nearly complete upon first pass.

Also noted is the lack of reliable clinical pharmacokinetic information on plasma levels of GTN and GDN metabolites with the maximum dose of the nitroglycerin ointment 0.4% (only 0.2% formulation was studied) and the inability to compare this directly (as in a relative BA study) or indirectly through literature or data in the public domain to levels produced with the RLD Nitro-Dur patch. Therefore, it is unclear whether plasma exposure to relevant species with the present product is within levels for which the Agency has found the RLD to be safe and effective for chronic use. The short duration of action with rectal application of this ointment, as well as lower total daily applied dose (3 mg) may result in daily area under the plasma concentration-time curve (AUC) levels of GTN within that of a patch providing a constant release of nitroglycerin. The RLD Nitro-Dur contains up to 160 mg nitroglycerin, delivering 0.8 mg/hr x 14 h = 11.2 mg/day (a 10-12h drug-free period recommended daily to prevent development of tolerance). However, the plasma GTN C_{max} of the intra-rectal ointment is likely to be significantly higher than that of the RLD patch.

The data provided in the original application indicated intra-anal application of 0.2% nitroglycerin ointment resulted in GTN C_{max} ~ 1 ng/mL and AUC 41 µg*min/L (equivalent to 41 ng*min/mL or 0.68 ng*h/mL). Comparison to the RLD Nitro-Dur is indirectly obtained through the NDA as well as published literature reports along with several other selected nitroglycerin-based products. The transdermal patches appear to provide general coverage for daily exposure to nitroglycerin but do not provide coverage for the GTN Cmax obtained with the 0.2% ointment and obviously the potential value using a 0.4% ointment. Several transmucosal products for acute use as well as prophylaxis appear to produce exposures in which GTN Cmax of the 0.4% ointment may be considered covered, however these were not used as RLD products and therefore cannot be used for support until such time as adequate patent certification and RLD designation by the Applicant is provided. It is also important to note that the table below is only related to the parent nitroglycerin and does not specifically address the support for the active GDN metabolites 1,2- and 1-3 dinitroglycerin which are less effective vasodilators than the parent but are present at higher levels than parent and have a circulating $t^{1/2}$ ~40 minutes compared with the very short circulating time of nitroglycerin $t^{1/2}$ ~ 1-3 minutes.

Nitroglycerin values cross selected products				
Product	**Reported Cmax (ng/mL)**	***Reported AUC value@***	**Converted AUC value (to ng*h/mL)**	**Comments**
Nitroglycerin Ointment (Rectogesic) 0.2%[a]	~ 1.0	*41 ng*min/mL*	0.7	Highest concentration is 2X (i.e. 0.4%)
Transdermal systems				
Nitro-Dur patch 0.4 mg/h[b]	N/A	*6100 pg*h/L*	6.1	Highest patch strength is 0.8 mg/h
Nitro-Dur 0.8 mg/h (Schering)[c]	~0.2	*2,050 pg*h/mL (AUC0-12h)*	2.1 (AUC0-12h) (~4.2 AUC0-24h)	
Nitroglycerin Transdermal Patch (Hercon Labs) 0.6 mg/hr[d]	0.5	*3,100 pg*h/mL*	3.1	
Nitrodisc 32 mg (0.4 mg/h)[e]	0.3	*3104 pg*h/mL*	3.1	
Transmucosal systems				
Nitrostat (sublingual) 0.6 mg[f]	~ 2	*14 ng*min/mL*	0.2	Labeled use indicates up to 3 tablets within 15 minutes may be necessary
Nitromist (oral lingual spray) 0.4 mg[g]	~0.8	*9.2 ng*min/mL*	0.15	Data is for 3 actuations (1.2 mg); Labeled use is for up to 3 sprays per attack

[a] Nitroglycerin ointment (Applicant study report); [b]Nitro-Dur patch: Sun et al., (1995) J Clin Pharmacol 35(4) 390-7; [c] NDA 20-145 (study #K89-2980-1); [d] ANDA 89-884; [e] NDA 20-146 review; [f] Nitrostat Product Information; [g] NDA review.

III. RECOMMENDATIONS

A. Recommendation on approvability

Though the original nonclinical review by the Division of Cardio-Renal Drug Products recommended approval, I concur with the current recommendation made by Dr. Leshin that this application not be approved until further comparative exposure data with approved nitroglycerin products for chronic use indicates exposure levels with the 0.4% ointment are within clinical historic use or additional nonclinical data is provided.

B. Recommendation for nonclinical studies

The recommendations are dependent on several factors including:

1) The ability to compel or otherwise obtain a relative bioavailability study of nitroglycerin ointment 0.4% under maximal conditions of use with that of the current Reference Listed Drug (RLD), Nitro-Dur, under maximal use conditions which demonstrates levels are within that of Nitro-Dur or, in the absence of above information, the ability of the Applicant to provide sufficient pharmacokinetic data from studies and/or the pubic domain which indicates plasma levels of GTN subsequent to maximal dosing of nitroglycerin ointment 0.4% application falls within pharmacokinetic levels of an approved and referencable nitroglycerin product approved for chronic use;

2) If exposure levels with nitroglycerin ointment 0.4% exceed that of the RLD(s), a determination that this represents a potentially significant increase in risk;

3) The clinical determination that the use of the product under the indication sought meet the criteria of a "chronic" use product under ICHS1A *Guideline for Industry: The Need for Long-Term Rodent Carcinogenicity Studies of Pharmaceuticals* which state:

    a. *Carcinogenicity studies should be performed for any pharmaceutical whose expected clinical use is continuous for at least 6 months (Note: it is expected that most pharmaceuticals indicated for 3-months treatment would also likely be used for 6 months. In an inquiry to a number of pharmaceutical research and regulatory groups, no cases were identified in which a pharmaceutical would be used only for 3 months.)*

    b. *Certain classes of compounds may not be used continuously over a minimum of 6 months but may be expected to be used repeatedly in an intermittent manner. It is difficult to determine and to justify scientifically what time represents a clinically relevant treatment period for frequent use with regard to carcinogenic potential, especially for discontinuous treatment periods. For pharmaceuticals used frequently in an intermittent manner in the treatment of chronic or recurrent conditions, carcinogenicity studies are generally needed. Examples of such conditions include allergic rhinitis, depression, and anxiety.*

Dr. Leshin has recommended toxicokinetic bridging studies be conducted in rat, mouse and rabbit in order to allow utilization of the carcinogenicity and reproductive toxicology studies described in the reference listed drug label. As these are largely dietary the degree of systemic exposure achieved by the parent compound is in significant question due to the extremely high first pass metabolism of nitroglycerin, this is particularly important as high dose levels described in the label may provide an overly optimistic and potentially inappropriate appearance of safety when compared with the doses used in clinical use. The same data without direct comparison with human levels for safety assessment is to be found

in apparently many if not all approved nitroglycerin products for use in acute or prophylaxis of angina.

Although I find the acceptability of such study utilization without a useful toxicokinetic bridge troubling, if the Applicant can demonstrate that their product does not provide greater exposure than approved nitroglycerin products as this would not represent a greater risk to the public I do not feel it is appropriate to require the Applicant to conduct a challenging bridging program to historical published data. If, however, the Applicant is unable to satisfy this request for comparative data or if the data indicates the product is not covered by approved products and represents an increased risk to the patient population, current Agency (b)(2) policy allows the Division to recommend conduct of missing or inadequate studies from the original RLD product – and in particular studies contributing to labeling. In this situation I agree with Dr. Leshin such bridging studies should be provided or the Applicant must present sufficient data from the public domain that would allow the Agency to more confidently express the relationship of the conditions of these studies to human use. If the clinical determination is that Chronic Anal Fissure treatment with nitroglycerin ointment would likely be recurrent use with total lifetime use > 6 months, an evaluation of carcinogenicity should be required if the Applicant is unable to demonstrate sufficient human exposure coverage from the historic carcinogenicity data described in the RLD label. This could entail the use of routes which avoid significant first pass metabolism in order to achieve significant nonclinical exposures to address human risk.

Additionally, it is noted that the local and systemic nonclinical safety information provided was of questionable value and plasma levels of GTN and GDN metabolites not generally obtained. There is also no nonclinical study of intra-rectal administration beyond 14-days duration. If the exposure levels with the current product exceed any chronic RLD it may be necessary to require the Applicant to generate additional nonclinical toxicology data, including potentially chronic toxicology studies, to support the maximum recommended human dose and resulting exposures unless the medical team considers current clinical safety data sufficient and/or the applicant is able to provide evidence of local and systemic safety for the maximum dosing regimen of the product from human experience worldwide.

Accordingly, the deficiency which should be communicated to the Applicant as part of a Complete Response letter is the following:

Deficiency 1: You have not adequately demonstrated that nitroglycerin ointment 0.4% use under conditions of maximal indicated dosing and administration methods produces plasma exposures of nitroglycerin and metabolites which are within that of the Reference Listed Drug, Nitro-Dur, nor other approved nitroglycerin products approved for repeated use for which you can use as a reference.

Information needed to address Deficiency 1:
You must provide evidence that under conditions of maximal administration as labeled your product produces exposure to nitroglycerin and metabolites that are within one or more Reference Listed Drugs approved for repeated or chronic use. Failure to provide this support in a future resubmission of the application will result in the following additional requirements:

- *Unless sufficient clinical experience is provided to support the local and systemic safety of the human exposure with maximum dosing regimen, repeat-dose toxicology studies using the intra-rectal route will be required in two species (one non-rodent) up to a chronic duration.*
- *Adequate nonclinical toxicokinetic bridging studies with dietary or topical exposure as appropriate to establish safety margins for animal reproductive toxicology and carcinogenicity studies described in the label.*
    - *Should bridging studies fail to establish adequate exposure to nitroglycerin and metabolites to support a risk assessment you will need to conduct new reproductive toxicology studies with routes that produce sufficient exposures to allow a risk assessment to be made.*
    - *Should bridging studies fail to establish adequate exposure to nitroglycerin and metabolites to support a risk assessment and you do not provide usage data or other persuasive argument to indicate this product should not be considered a chronic use (i.e. >6 months lifetime usage) product you will need to conduct a carcinogenicity evaluation with routes that produce sufficient exposures to allow a risk assessment to be made.*

C. Recommendations for labeling:
None. Labeling is not being undertaken at this time.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-21359	ORIG-1	PROSTRAKAN INC	CELLEGESIC NITROGLYCERIN OINTMENT 0.4%

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

/s/

ADAM M WASSERMAN
03/13/2010

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	**21-359**
Supporting document/s:	SD-53
Applicant's letter date:	Sept 30, 2009
CDER stamp date:	Sept 30, 2009
Product:	**Nitroglycerin Ointment 0.4%**
Indication:	**Chronic anal fissure pain**
Applicant:	**ProStrakan International Limited**
Review Division:	Division of Anesthesia, Analgesia and Addiction Products
Reviewer:	L. Steven Leshin, DVM, PhD
Supervisor/Team Leader:	Adam Wasserman, PhD
Division Director:	Bob A. Rappaport, MD
Project Manager:	Chris Hilfiger

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

From the PharmTox perspective, the application should not be approved.

1.1.2 Additional Non Clinical Recommendations

Additional nonclinical studies are recommended below. If the application is not approved this cycle, the Applicant must submit the requisite studies and updated nonclinical information pertinent to labeling in the resubmission.

Additional studies include:

1) Bridging studies to establish the toxicokinetic exposure parameters when nitroglycerin is administered in the diet of the rat and mouse as in the carcinogenicity studies.

2) Bridging studies to establish the toxicokinetic exposure parameters when nitroglycerin is administered in the diet and topically as in the rat and rabbit reproductive and developmental studies.

If sufficient evidence provided by the above studies or scientific justification cannot be established that would allow the reliance of this product on carcinogenicity and reproductive and developmental safety of the reference listed product, then specific studies will be necessary to address the safety in these areas. These would include:

3) Carcinogenicity studies in two species, a 2-year daily dosing study in the rat, and either a 2-year daily dosing study in the mouse or a 6-month study in an appropriate transgenic mouse model.

4) Reproductive and Developmental Toxicology studies to address
    a) Fertility and early embryo development
    b) Embryo-fetal development
    c) Pre- and post-natal development

A special situation exists for carcinogenicity studies, in that if the effective product would not be used chronically (for greater than 6 months), or intermittently on a continuous basis (for an expected total duration of use of more than 6 months; Guidance for Industry: ICH-S1A), then carcinogenicity studies would not be necessary. It is anticipated based on the current state of

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knowledge of anal fissures and their recurrence, that this product, if approved, would relieve the pain of anal fissures, but not affect healing, and would likely be used intermittently and continuously. Therefore, if reliance by the 505(b)(2) process for carcinogenicity studies is inappropriate for this product, The Applicant will need to provide justification that the potential use of their product in this disease would not be chronic or intermittently on a continuous basis, otherwise a carcinogenicity studies in 2 species will be required.

Explanation for the requested additional studies:

Although this application is a 505(b)(2), relying on the Agencies determination of safety from the Reference Listed Drug, there is insufficient nonclinical information to support the administered dose of 0.4% nitroglycerin. As best as this reviewer can determine, the clinical systemic exposure would be expected to be greater than any currently approved and marketed nitroglycerin preparations, except for IV formulations that are used during (cardiovascular) surgery. While the human pharmacokinetic data provided in the NDA is similar to that for other nitroglycerin formulations, the clinical dose of 0.4% nitroglycerin is now two-fold greater that used for the pharmacokinetic study, 0.2% (Study NTG 98-02-02). The expected nitroglycerin and metabolite exposure is likely two-fold that of the approved products referenced through the 505(b)(2) process. The enhanced bioavailability of nitroglycerin with rectal administration (50% bioavailability) probably explains the high incidence of the major adverse event, headache, observed in clinical trials (e.g., incidence in Study REC-C-001: 0.4% nitroglycerin 70%, placebo 48%) despite the application of a lower amount of topical nitroglycerin than used for cardiovascular-related indications.

Past nonclinical studies relied mostly on nitroglycerin mixed into the diet with no determination of systemic or tissue exposure. Since nitroglycerin is rapidly metabolized upon oral and dermal application, it is unknown if the nonclinical studies referenced by the 505(b)(2) pathway support the safety of this product for the proposed dose. The Applicant has not provided scientific justification for the reliance on the referenced product Nitro-Dur to support their product's safety. By obtaining nonclinical pharmacokinetic data, the bridging studies will allow a determination of the appropriateness of relying on those studies.

Internal Recommendation to Clinical Pharmacology

In conjunction with the requested nonclinical data, we recommend that the Clinical Pharmacology Reviewers ask for the appropriate clinical pharmacokinetic data for the 0.4% nitroglycerin ointment (perhaps by conducting a new study or appropriate pharmacokinetic modeling). The only pharmacokinetic data for this product is from a study with the 0.2% ointment, an earlier formulation that was eventually demonstrated to be clinically ineffective. The pharmacokinetics of the 0.2% ointment was approximately similar to the numerous approved topical, oral, and sublingual nitroglycerin products, but the 0.4% ointment is expected to

exceed these values, resulting in exposures unsupported by the nonclinical studies and most importantly unsupported by the reference listed drug of this 505(b)(2) application.

1.1.3 Labeling

Labeling changes will be reviewed when the application is closer to being approved. It was noted in the initial NDA review of 2002 that the label should be consistent with class-labeling used for other nitroglycerin containing products. Since that time there have been a number of changes to labeling policy including the Physician Labeling Rule (PLR) and Structured Product Labeling (SPL) format. In the April 17, 2009 response to pre-submission questions, the Division requested the Applicant update nonclinical information for the period from 2002 through the 2009 resubmission that is pertinent to labeling. The applicant did not provide any additional information in the present submission.

1.2 Brief Discussion of Nonclinical Findings

Most of the nonclinical studies in support of this NDA were obtained from the published literature and reviewed by Dr. A.G. Proakis (DARRTS date April 10, 2002, Division of Cardio-Renal Drug Products). With the exception of a few newer nitroglycerin pharmacology papers referenced with the clinical study, there has been no new nonclinical information submitted since the original application was reviewed. The original nonclinical review of 2002 recommended approval.

Nonclinical toxicology support for this application is mostly based on approval of past nitroglycerin products via the 505(b)(2) pathway. However, this application for rectal administration is a new route of administration. The Applicant also justified safety by noting the total proposed dose of nitroglycerin ointment 0.4% applied intra-anally twice daily is 3.0 mg per day which is less than the recommended topical dose of 6-30 mg nitroglycerin applied every four to eight hours of the approved topical 2% nitroglycerin ointment for treating angina pectoris. However, since the tissues involved in local absorption and toxicity, as well as the bioavailability of nitroglycerin are quite different between anorectal and topical (dermal) administration, this applied dose comparison is inappropriate. Nonclinical studies in rabbits were conducted to support the safety of this route of administration.

Local Toxicology: Studies in rabbits were conducted to support the safety of the rectal route of administration. There was minimal anorectal irritation in rabbits administered the formulation with or without nitroglycerin. Rabbits received twice daily applications at an 8 hour interval, of 100 µL of 0.2% or 0.4% nitroglycerin ointment for 14 days (Chandler 2001: X1F237G). The amount of applied nitroglycerin was not provided and there was insufficient information to calculate the amount applied. Control groups included placebo, 100% petroleum jelly and no treatment. Histological assessment of epithelium, leukocyte infiltration,

vascular congestion and edema, were scored 0 to 4 corresponding to increasing severity, then summed together (possible score of 0 to 16). Tissues examined included the anal canal, rectum, distal colon, and keratinized perianal skin. The anorectal irritation index was 1.3 and 2.2 for 0.2% and 0.4% nitroglycerin, respectively, and for controls ranged from 2.2 to 3.2, indicative of minimal irritation in all groups. Similar results were found in an earlier 5-day study in rabbits treated with formulation without nitroglycerin (McCartney 1998: X8D225).

The local toxicity for anorectal administration was inadequately conducted. The mg amount of nitroglycerin applied was not stated, and missing information did not allow an estimate of the mg amount applied. The 14-day duration of application was inadequate. An early clinical study was conducted for 14 days, but later phase 3 clinical studies were conducted for 21 or 56 days. In addition, the nonclinical study only examined behavioral and observational health, anorectal gross and histopathology, but not clinical pathology, nor gross and histopathology of other tissues and organs. There was no determination of pharmacokinetics.

Systemic Toxicology: The bioavailability with rectal administration is much greater than with oral (lingual or sublingual products) or dermal applications in humans. There are no toxicokinetics or pharmacokinetics data from the submitted published and unpublished nonclinical repeat-dose rectal toxicology studies. Many of these nonclinical studies were conducted with dietary administration, and it is unlikely that sufficient nitroglycerin was administered to make an accurate assessment of systemic safety based on a comparison with human pharmacokinetic information. This is due to the rapid metabolism of dietary or orally administered nitroglycerin. However, it is unclear if nonclinical studies with appropriate dosing are feasible. In rat and dog studies, methemoglobinemia and cyanosis occurred at high doses (in rats with nitroglycerin mixed into the diet at ≥1%, and in dogs administered oral capsules of doses ≥100 mg/kg/day; Ellis 1984). Methemoglobinemia results from the metabolism of nitroglycerin to nitrites which oxidize oxyhemoglobin to methemoglobin. Although nonclinical support for clinical safety with rectal application is lacking, clinical safety has relied on the experience of nitroglycerin use for over a century in the treatment of cardiovascular-related illnesses.

Pharmacokinetics/Toxicokinetics: Submitted single-dose bioavailability studies in rats indicated rectal administration resulted in bioavailability of 27-84% compared to <2% with oral administration. Human rectal bioavailability was approximately 50%. Pharmacokinetics for the bioavailability study of rectally administered nitroglycerin in the rat is presented in Table 1. Since there is no toxicology associated with this or other bioavailability studies, the data are not useful for addressing either local or systemic safety. The nitroglycerin toxicokinetics in the published toxicological studies is unknown since they were conducted at a time that toxicokinetic data was not usually obtained and bridging studies to obtain this information have not been conducted. The lack of nonclinical toxicokinetic

data and lack of human pharmacokinetic data for the 0.4% nitroglycerin drug product does not allow a determination of safety margins.

Table 1: Interspecies Pharmacokinetic Parameters for Nitroglycerin

Species / Study	Total nitroglycerin applied / day	Route	C_{max} (ng/mL)	AUC (ng*min/mL)
Rat (bioavailability study) (Kamiya et al 1982. j Pharm Sci 71:621-624)	1.7 mg/kg 3.5 mg/kg	rectal	0.1 ± 0. 6 0.2 ± 0.2	2.2 ± 0.7 4.0 ± 3.6
Bioavailability was shown to be dependent on the location from the anus of the administered nitroglycerin, 3.5 mg/kg dose rectally instilled without restriction yielded a mean bioavailability of 26.7 ± 7.0%, the same dose applied to a restricted distance within 3.5 cm from the anus yielded an 83.5 ± 74.5% bioavailability, and a 1.75 mg/kg dose applied to a restricted distance within 2.0 cm from the anus yielded a bioavailability of 91.2 ± 30.4%. Oral dosing produced a bioavailability of 1.8 ± 0.9%.				
Human Study NTG 98-02-02	0.2% ointment qd (0.75 mg) tid (2.25 mg)#	rectal	0.2-1.0* 0.2-0.6*	41 ± 19 42 ± 9
	0.3 mg (10 µg/mL at 1 mL/min infusion over 30 min	IV	0.2-0.3*	
* estimated from Figure 1 in the Clinical Pharmacology Review of March 2002 # tid was administered for 7 doses, PK was determined after the 7th dose Concentrations in the multiple dose study were unmeasurable prior to the seventh dose, thus, the concentration time profile of multiple dose looks like that of single dose.				
Reference Drug* **Human, NDA 87-496 Nitro-Dur Transdermal Infusion System**	75 mg (10 cm^2 of 26 mg nitroglycerin / 5 cm^2)	dermal	0.99 ± 0.2# Css 0.9 ± 0.2	19.5 ± 4.4 mg-hr/mL
* Nitro-Dur (NDA 20-145, approved Dec 1985, Key Pharm) original results unavailable # Tmax at 18 hours, Css attained at 5 hours				

Carcinogenicity: The degree of risk to humans regarding carcinogenesis based on non-clinical toxicological findings, hepatocellular carcinoma and testicular tumors, is difficult to ascertain, although nitroglycerin has been used to treat angina for over a century. The assessment of these effects in rodents was conducted with dietary administration of nitroglycerin in which the drug encounters near-complete hepatic first-pass metabolism. Lacking toxicokinetic data over the 24 hour daily availability of the nitroglycerin-containing diet (rats eat in multiple small bouts, tending to be synchronized to 'lights-off' when adapted to typical housing conditions). The assessment of nitroglycerin's carcinogenicity potential from topical dermal or rectal application, has not been conducted.

Reproduction and Developmental Toxicity: As indicated above with carcinogenic effects, the risk to humans of the nonclinical findings, infertility due to

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aspermatogenesis in second and third generation rats, could not be determined do to the inability to relate the nonclinical findings with dietary or topical administration to clinical exposure.

The Applicant did not include the necessary label Labor and Delivery section. They will need to provide this section if they resubmit the application. In recent years, nitroglycerin has been used off label as a tocolytic to prevent or slow premature labor, so there is some pertinent clinical information available. This product's indicated route may be the optimal route for this purpose. The Applicant will need to address current information about the product's use in labor and delivery if the application is resubmitted. The Labor and Delivery section is also missing in other nitroglycerin product labels. The label will be consulted to the Maternal Health Team in the Pediatric and Maternal Health Staff when labeling becomes appropriate.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number (Optional)

55-63-0

2.1.2 Generic Name

nitroglycerin

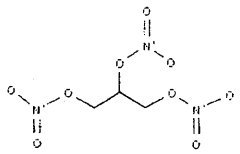
2.1.4 Chemical Name

1,2,3-propanetriol trinitrate
1,3-dinitrooxypropan-2-yl nitrate (IUPAC name)

2.1.5 Molecular Formula/Molecular Weight

$C_3H_5N_3O_9$ / 27.09

2.1.6 Structure



2.1.7 Pharmacologic class

Vasodilator; organic nitrate

2.2 Relevant IND/s, NDA/s, and DMF/s

NDA 20-145 (Nitro-Dur; Key Pharmaceuticals, Inc; Approved 1985; Reference listed drug)
IND 45,326 (nitroglycerin (b) (4) Cellegy Pharmaceuticals, now ProStrakan Inc.; (b) (4))
DMF (b) (4) (nitroglycerin ointment 0.4% w/w , (b) (4)

2.3 Clinical Formulation

Table 2 : Composition of Nitroglycerin 0.4% Ointment

Ingredients	Quantity % (w/w)	Function	Complies with
Active Ingredient			
(b) (4) (Nitroglycerin, USP (b) (4) Nitroglycerin (b) (b) (4)	4.0	Active	USP
Excipients			
White Petrolatum	(b) (4)		USP
Lanolin			USP
Paraffin			NF
Sorbitan Sesquioleate			NF
			(b) (4)

2.4 Proposed Clinical Population and Dosing Regimen

Topical ointment is to be applied within the anal canal (375 mg of ointment, equivalent to 1.5 mg of nitroglycerin) approximately every 12 hours.

The total proposed dose of nitroglycerin for Cellegesic NTG ointment 0.4% applied intra-anally is 3.0 mg per day.

Current Reviewer's comment: This is less than the recommended topical dose of 6-30 mg nitroglycerin transdermal ointment every four to eight hours for treating angina pectoris, but due to enhanced bioavailability of rectal administration, the total systemic exposure may be greater with the 0.4% nitroglycerin product. There is no pharmacokinetic data for the 0.4% nitroglycerin product.

2.5 Regulatory Background

Cellegy Pharmaceuticals Inc. originally submitted NDA 21-359 for Anogesic™ Ointment 0.4% on June 26, 2001 to the Division of Cardio-Renal Drug Products as a 505(b)(2) application. The product tradename was later changed to Cellegesic™, but this name was denied Dec 29, 2009, and another name has recently been submitted for review, Rectogesic. This 505(b)(2) submission references Nitro-Dur (NDA 20-145, a transdermal film extended release nitroglycerin product). The original NDA for Cellgesic was withdrawn in April 2002 due to failed efficacy studies and problematic analysis. Another study, CP125 03-02-01, and analysis plan was agreed upon in a meeting with the Division on January 31, 2003. The NDA was resubmitted in June 30 2004 and was determined on Dec 23, 2004 to be not approvable due to insufficient information about the clinical trial. In response, the NDA was resubmitted again on April 15, 2005, but responses were again inadequate as outlined in the Dec 23, 2004 Discipline Review letter and July 7, 2006 Approvable letter in which another study would be necessary and the past clinical deficiencies associated with the drug development program would need to be addressed. In this Sept 30, 2009 Complete Response, study CP125 03-02-01 is submitted to address the previous clinical deficiency. However, the 12-month follow-up phase of this study is still ongoing.

The Pharmacology/Toxicology Review was completed by Dr. A.G. Proakis in March 2002, with the product recommended for approval after the label was made consistent with other approved nitroglycerin products. With the June 2004 resubmission, the review filed in Aug 2004 indicated there was no new Pharmacology/Toxicology information provided.

In Nov 2006, the NDA ownership was transferred from Cellegy to Strakan Pharmaceuticals Ltd (later became ProStrakan). The Application was transferred to DAARP on March 15, 2007 and the Division discussed with the Applicant resubmission of the application in May 2007. At this meeting the Division agreed with the previous FDA conclusions from DCRP that Studies 98-02-01, 00-02-01, and 03-02-01 failed to show efficacy of Cellegesic as a treatment for pain due to anal fissures, and that an additional study was necessary to support efficacy of Cellegesic for the proposed indication.

In the April 17, 2009 response to pre-submission questions, Pharmacology-Toxicology requested that the Applicant provide updated information from 2002-present related to the mutagenicity, carcinogenicity, fertility, pregnancy and lactation labelling sections. However, no additional pharmacology-toxicology information was provided in the Complete Response.

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3 Studies Submitted

3.1 Studies Reviewed

No nonclinical studies were submitted.

3.3 Previous Reviews Referenced

1) NDA 21-359 PharmTox review of April 10, 2002
2) NDA 21-350 PharmTox review of Aug 9, 2004

4 Pharmacology

Refer to previous reviews indicated in section 3.3.

5 Pharmacokinetics/ADME/Toxicokinetics

Refer to previous reviews indicated in section 3.3.

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6 General Toxicology

Refer to previous reviews indicated in section 3.3.

Table 3: Summary of Mutagenicity Studies (based on previous reviews)

Study type/ Reference	Methods	Result
Mutagenicity		
Bacterial Mutagen (Ames) Test CM Maragos et al, 1993. Mut Res; 298:187-195	*S. typhymurium* tester strains (TA1975, TA102, TA1535, TA1538, TA100, TA100NR and YG1026) **TA1535:** NTG concentrations ≥2.5 ug/plate resulted in dose-dependent greater than 2-fold increases in the number of revertant colonies in the absence or presence of metabolic activation. **Others:** Negative mutagenicity of NTG was manifest only in the strain (TA1535) in which reversion occurs by single base pair substitution of G-C in the hisG46 marker (Mortelmans and Zeiger 2000, Mut Res 455:29-60.)	**Positive** (only in strain TA1535) • a C to T mutation was found, • similar to studies in which nitric oxide mediates mutagenicity
Dominant lethal mutation test Ellis 1978 Midwest Research Institute Progres Report No 8. for US Army Medical Research;	male rats treated with NTG in the diet (0.0, 0.01, 0.1 or 1% NTG) for 13 weeks were mated with untreated females. NTG treatment had no adverse effects on fertility, preimplantation loss or post implantation loss.	**Negative**
Clastogenicity		
in vivo exposure 13-weeks, rat bone marrow cell cytogenetic assay Ellis 1978 Midwest Research Institute Progres Report No 8. for US Army Medical Research;	rats in general tox study fed approx 230 mg/kg/day of nitroglycerin mixed in diet. Peripheral blood lymphocytes and kidney cells were cultured, treated with NGT activated, arrested, processed, stained and examined for both numerical and morphological chromosome aberrations.	**Negative**
in vivo exposure 2-years, rat bone marrow cell cytogenetic assay Ellis 1978 Midwest Research Institute Progres Report No 8. for US Army Medical Research;	rats in general toxicology study for 2 years fed nitroglycerin 363 mg/kg/day in males and 434 mg/kg/day in females. Bone marrow cells and kidney cells were obtained from rats at the end of the 2-year toxicity (carcinogenicity) study and cultured. Actively dividing kidney cell cultures, bone marrow cells and phytohemagluttin-stimulated lymphocytes were arrested at metaphase with colchicine treatment and fixed onto slides for	**Negative**

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	examination of chromosome morphological aberrations. NTG treatment (363 mg/kg/day in males and 434 mg/kg/day in females) produced no significant chromosomal aberrations in this study	
dog lymphocytes cytogenetic assay Ellis 1978 Midwest Research Institute Progres Report No 8. for US Army Medical Research;	dogs in general toxicology study for 13 weeks administered approx 5 mg/kg/day nitroglycerin in capsules. Peripheral blood lymphocytes and kidney cells were cultured, treated with NGT activated, arrested, processed, stained and examined for both numerical and morphological chromosome aberrations. NTG treatment caused no significant increase above control frequency of chromosomal aberrations.	**Negative**
in vitro, Chinese Hamster Ovary cells (CHOK1)	NTG concentrations of 50 and 145 ug/ml that caused 65% and 99% cytotoxicity, respectively, did not induce cell mutations. This assay was not conducted in the presence of metabolic activation conducted only in the absence of metabolic activation	**Negative**
Applicant suggests that genetic repair mechanisms *in vivo* may blunt the potential mutagenic effects of nitroglycerin.		

Table 4: Summary of Carcinogenicity Studies (based on previous reviews)

Study type/ Reference	Methods	Result
Rat Ellis et al, 1978 Midwest Research Institute Progres Report No 8. for US Army Medical Research; Ellis et al, 1984 Fund Appl Tox 4:248-260	diet, 434 mg/kg/day (high dose)	**Dose-related effects:** • **Liver: fibrotic and neoplastic changes** • **Hepatocellular carcinoma** **males 62% *vs* 0% in controls** **females 44% vs 0% controls** • **Testes: interstitial cell tumors** **52% vs 8% in controls**
	control: 0.0% males females	incidence of hepatocellular carcinomas 0 of 24 males 0 of 29 females
	0.05% in diet, males 3.04 mg/kg/day females 3.99 mg/kg/day	NOAEL
	0.1% in diet males 32.5 mg/kg/day females 38.1 mg/kg/day	incidence of hepatocellular carcinomas 3 of 26 males 2 of 28 females
	1% in diet males 363 mg/kg/day females 434 mg/kg/day	incidence of hepatocellular carcinomas 13 of 21 males 11 of 25 females testicular interstitial cell tumors cholangiofibrosis in males and females

from Ellis 1984:

TABLE 6

HISTOPATHOLOGICAL LESIONS IN RATS FED TNG FOR 24 MONTHS AND THOSE DYING AT UNSCHEDULED TIMES

	Dose (% in feed)							
	Males				Female			
Lesion	0	0.01	0.1	1	0	0.01	0.1	1
Cholangiofibrosis	0/24	0/28	0/26	18/21	0/29	0/32	0/28	24/25
Hepatocellular carcinoma and/or neoplastic nodules	1/24	0/28	4/26	15/21	0/29	1/32	3/28	16/25
Extensive splenic pigmentation	3/24	1/28	6/26	7/21	1/29	2/32	5/28	16/25
Excessive renal epithelium pigmentation	1/24	0/28	0/26	11/21	0/29	3/32	4/28	31/25
Interstitial cell tumors of testis	2/24	1/28	3/26	11/21	—	—	—	—
Mammary gland tumors	1/24	0/28	1/26	0/21	13/29	19/32	12/28	2/25
Pituitary chromophobe adenoma	14/23	12/25	14/25	5/19	22/28	21/32	21/27	7/24

Note. Data are rats with lesions/rats with readable slides.

Mouse Ellis et al, 1978 Midwest Research Institute Progres Report No 8. for US Army Medical Research; Ellis et al, 1984 Fund Appl Tox 4:248-260	diet, 0.5% NTG females up to 1058 mg/kg/day, males up to 1022 mg/kg/day	**Not carcinogenic**

Mechanism of NTG associated hepatic carcinogenesis	
Rat Tamano et al, 1996. Carcinogenesis; 17:2477-2486	K*ras* point mutations in some of the hepatic tumors Also occur with nitric oxide-induced DNA damage in bacteria Suggesting a role for nitric oxide generation in NTG induced carcinogenesis Male F344 rats received a single intragastric dose (1.2 g/kg) of NTG at 6 weeks of age and/or a diet containing 1% NTG from 8 weeks until necropsy (for up to 78 weeks). Some animals were subjected to 2/3 partial hepatectomy at 9 weeks of age. Five sequential sacrifices (14, 32, 52, 78 and 84 weeks of age) were performed. • No liver tumors developed in control rats or in rats receiving a single intragastric dose of NTG, even when dosing was followed by partial hepatectomy. • Preneoplastic foci, mainly clear cell and mixed cell type, were found from 14 weeks of age in rats receiving NTG in the diet. Focal eosinophilic areas (atypical foci) composed of atypical hepatocytes that often extended into the veins were observed beginning at 52 weeks of age. • Some mixed hepatocholangiocellular adenomas and carcinomas arose in eosinophilic lesions. Hepatocellular carcinomas were seen beginning at 78 weeks of age, but only in rats receiving dietary NTG. • The incidence of hepatocellular carcinoma in the latter animals was >50%. Most hepatocellular carcinomas were well differentiated. • The carcinogenic effect of NTG given in the diet was not affected by prior administration of a large single dose followed by partial hepatectomy. • No *p-53* mutations were found in 18 tumors, but K-*ras* point mutations were found in 8/18 tumors, mostly those of cholangiocellular elements. The investigators conclude that while these mutation types have also been commonly seen in bacteria after nitric oxide-related DNA damage, the fact that the tumors arose only on prolonged exposure to a large dose of this agent seems consistent with a more complex mechanism involving genetic and/or epigenetic effects of NTG

Table 5: Summary of Reproductive and Developmental Studies (based on previous reviews)

Study type/ Reference	Methods	Result
3-generation reproduction study CD Rat Ellis et al, 1978 Midwest Research Institute Progres Report No 8. for US Army Medical Research;	dietary administration of NTG Parents and offspring of each generation received diets containing 0.01%, 0.1% or 1% NTG (treatment continued through successive F1 and F2 generations). control: 0.0% males females 0.05% in diet, males 3.04 mg/kg/day females 3.99 mg/kg/day 0.1% in diet males 32.5 (31.5?) mg/kg/day females 38.1 mg/kg/day 1% NTG, average NTG intake males 363 mg/kg/day females 434 mg/kg/day	**severely impaired fertility in F1 and F2 high dose males**, small testes, decreased spermatogenesis • **Most parameters of reproductive performance (litter size, live-born index, birth weight viability and weaning weight), were lower than control in the high dose F1 and F2 litters.** • **These adverse effects of the high dose of NTG appeared to be secondary to the poor nutritional status of the dams. The feed intake of these high dose rats was about 65% of control.** **fetuses:** • increased incidence of incomplete ossification of the hyoid bone • nonsignificant higher than control incidence of diaphragmatic hernia • no other evidence of NTG-related teratologic effect
	F_0 generation 10 males and 20 females from each dose group were mated after receiving the test diets for 6 months. Each male was housed with 2 females from the same dose group for 14 days. For assessment of NTG induced malformations, the females of the F0 generation were mated for a third time with untreated males.	Fetal weight among offspring from treated F0 females was comparable to control. **Soft tissues:** examined one-half of the fetuses of the F0 generation only a single malformation, diaphragmatic hernia, in the high dose group that appeared to be related to NTG treatment (occurred in 4 of 19 litters vs. 0 of 15 for control, not statistically significant). **Skeletal:** examined remaining one-half of the fetuses higher than control incidence of incomplete ossification of the hyoid bone among high dose fetuses. No effect on sternabra, centrum and bones of the skull
	F_1 Generation The offspring from the first mating (F1a, first litters) were discarded at weaning, The offspring from the second mating (F1b, 20 to 24 of	severely impaired fertility in F1 high dose males The first mating of the F1 high dose rats produced only 3 F2a.litters. The second mating of the F1 generation resulted in less

	each sex) within the same dosage group were mated for 14 days at 3 months of age.	than a 50% incidence of pregnancy.
	F_2 Generation The F2a generation was discarded at weaning. The F1b males and females were mated for a second time to produce the F2b generation. Males and females from the F2b generation were mated twice beginning at 3 months of age according to the same procedure and the study was terminated upon weaning of the F3b pups.	• severely impaired fertility in F2 high dose males • Although 14 pairs of F2a rats were mated twice, there was only one F3 litter. When F2a high-dose females were mated with control rats, 13 of 14 rats became pregnant and produced the F3 generation. • Microscopic examination of high dose F2 males revealed severe aspermatogenesis and mild to moderate increased interstitial tissue in the testis. • The F2 males had very small testes and that they produced a high incidence of vaginal plugs without sperm. • The poor reproductive performance of the F2 generation was likely due to an adverse effect on male fertility.
Embryo-Fetal Development **Rat** Skutt et al, 1985. Shinyaky Rinsho 34:2009-2023.	2% NTG ointment applied topically to the shaved backs of pregnant CD rats on days 7 through 17 of pregnancy 1, 2 and 4 gm/kg/day = 20, 40 and 80 mg NTG/kg/day; for 6 hours control rats received 4 gm/kg/day of non-NTG containing ointment 36/dose group After 6 hours the excess ointment was removed to avoid oral intake of the product. day 20 of pregnancy 2/3 of the dams were sacrificed and the remaining dams were allowed to litter and then sacrificed 21 days after giving birth	No adverse effects on length of gestation, nesting and nursing behavior of the dams or on the appearance and behavior of the pups. **No treatment related malformations or variations.**
Embryo-Fetal Development **Rabbit** Imoto, 1986. J Toxicological Sciences 11: 59-70.	New Zealand white rabbits treated topically with 10% NTG or placebo ointment on days 6 through 18 of pregnancy ointment applied to the shaved backs of rabbits was equivalent to 0 (untreated), 0 (placebo), 15, 60 and 240 mg NTG/kg/day (14-16/dose group); the application site was covered with polypropylene film to prevent	Red spots were noted on the skin of placebo and NTG treated dams but not on untreated controls. **NTG treatment had no adverse effect on dams.** **No treatment related malformations or variations.**

	ointment removal and remained on the animals for 20hrs/day. On day 29 the animals were sacrificed	
Embryo-Fetal Development **Rabbit** Miller et al, 1985. Shinyaku Rinsho 34:2024-2032	pregnant New Zealand white rabbits, groups of rabbits were treated topically with 2% NTG ointment (1, 2 or 4 gm of ointment providing 20, 40 or 80 mg NTG/kg/day) or placebo on days 6 through 18 of pregnancy. caesarean section performed on day 29 of pregnancy	**No treatment related malformations or variations.**

11 Integrated Summary and Safety Evaluation

Refer Section 1.2 and to previous reviews indicated in section 3.3.

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-21359	ORIG-1	PROSTRAKAN INC	CELLEGESIC NITROGLYCERIN OINTMENT 0.4%

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

/s/

LAWRENCE S LESHIN
03/09/2010

ADAM M WASSERMAN
03/09/2010

Memorandum

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF CARDIO-RENAL DRUG PRODUCTS

DATE: 8/04/04

FROM: Anthony G. Proakis, Ph.D., Pharmacologist, HFD-110

THROUGH: Charles A. Resnick, Ph.D., Supervisory Pharmacologist, HFD-110

TO: Norman Stockbridge, M.D., Ph.D., Acting Director, Division of Cardio-Renal Drug Products, HFD-110
Darrell Allis, Regulatory Health Project Manager, HFD-110

SUBJECT: NDA 21,359 (Cellegesic™ Nitroglycerin Oint. 0.4%, Cellegy Pharmaceuticals, Inc.)

NDA Resubmission Date: 6/30/04
Center Receipt Date: 7/01/04

BACKGROUND

Cellegy Pharmaceuticals, Inc. originally submitted NDA 21,359 for Cellegesic™ Nitroglycerin Oint. 0.4% on June 26, 2001 but the NDA was withdrawn in April 2002 in order for the sponsor to carry out another clinical study. The non-clinical pharmacology and toxicology studies that were included in the June, 2001 original submission were reviewed (Pharmacology/Toxicology Review, 3/14/02). The product was deemed approvable from a non-clinical perspective provided that statements in the sponsor's draft labeling that refer to results of animal toxicity studies be made consistent with labeling used for other nitroglycerin containing products. The resubmission of NDA 21,359 contains no new non-clinical pharmacology and toxicology studies requiring review.

RECOMMENDATIONS

NDA 21,359 (for Cellegesic™ Nitroglycerin Oint. 0.4%) remains approvable from a non-clinical perspective. Recommended changes to the sponsor's draft labeling can be found in the attached Pharmacology/Toxicology Review (3/14/02).

Attachment: NDA 21,359, Pharmacology/Toxicology Review (3/14/02)

NDA 21,359/Files
HFD-110/Proj.Mgr.
HFD-110/CResnick
HFD-110/AProakis

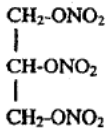
Reference ID: 2969428

PHARMACOLOGY/TOXICOLOGY REVIEW COVER SHEET

NDA number: 21,359
Review number: 1
Date of submission: 6/22/01 (Sponsor's letter date); 6/26/01 (Center receipt date)
Information to sponsor: Yes ( ) No (X)
Sponsor : Cellegy Pharmaceuticals, Inc.
Manufacturer for drug substance: Nitroglycerin (b) (4) in (b) (4), USP is manufactured by (b) (4)

Reviewer: Anthony G. Proakis, Ph.D.
Division: Cardio-Renal Drug Products
HFD #: 110
Review completion date: 3/14/02
Drug Product: CELLEGESIC Ointment (0.2% and 0.4% w/w nitroglycerin)
Drug:

Generic name: Nitroglycerin
Code name: None
Chemical name: 1,2,3,-propanetriol trinitrate
CAS registry number: 55-63-0
Mole file number:
Molecular formula/molecular weight: $C_3H_5N_3O_9$ / 227.09
Structure:



Relevant IND: Cellegy Pharmaceeuticals IND # 45,326 (IND under which clinical trials were conducted)

Drug Class: Vasodilator

Indication: Relief of pain associated with chronic anal fissure.

Clinical Formulation:

Ingredient	% w/w (0.2%)	% w/w (0.4%)	Function
(b) Nitroglycerin ir (b) (4) USP	2.0	4.0	Active Ingredient
White Petrolatum, USP	(b) (4)		
Lanolin, USP			
Propylene Glycol, USP			
Paraffin, NF			
Sorbitan Sesquioleate, NF			

Route of Administration: Applied topically to the anal canal.

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Proposed dosage regimen: A dose of approximately 374 mg of ointment (obtained with a dose-measuring device) is to be applied 2 or 3 times daily until anal pain is gone or the fissure is healed. The amount of NTG contained in the 374 mg dose delivered from the 0.2% ointment is 0.75 mg and from the 0.4% ointment is 1.5% NTG.

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

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Executive Summary

I. Recommendations

A. Recommendation on Approvability

This product is approvable from a non-clinical perspective.

B. Recommendation for Nonclinical Studies

None

C. Recommendations on Labeling

Statements in the labeling that refer to results of animal toxicity studies should be consistent with class-labeling used for other nitroglycerin containing products. Accordingly, the labeling for CELLEGESIC ointment should read as follows:

Under PRECAUTIONS, Carcinogenesis, Mutagenesis, Impairment of Fertility:

Rats receiving dietary nitroglycerin for 2 years developed dose-related fibrotic and neoplastic changes in liver (including carcinomas) and interstitial cell tumors in testes. At the highest dose tested (434 mg/kg/day), the incidence of hepatocellular carcinoma was 62% *vs* 0% in controls for males and 44% *vs* 0% in controls for females, and the incidence of testicular tumors was 52% *vs* 8% in controls. Lifetime dietary administration of up to 1058 mg/kg/day of nitroglycerin was not tumorigenic in mice.

Nitroglycerin was weakly mutagenic in Ames tests performed in two different laboratories, but there was no evidence of mutagenicity in an *in vivo* dominant lethal assay with male rats treated with doses up to about 363 mg/kg/day, p.o., or in *in vivo* cytogenetic tests in rat bone marrow cells and dog lymphocytes. Nitroglycerin was not mutagenic *in vitro* in a Chinese Hamster Ovary assay which was conducted in the absence of metabolic activation.

In a three-generation reproduction study, rats received dietary nitroglycerin for six months prior to mating of the F_0 generation, with treatment continuing through successive F_1 and F_2 generations. The highest dose (434 mg/kg/day) was associated with decreased feed intake and body weight gain in both sexes of all generations. No specific effect on the fertility of the F_0 generation was seen. Infertility noted in subsequent generations was attributed to aspermatogenesis in the high-dose F_1 and F_2 males.

Daily topical application of nitroglycerin ointment to the skin of pregnant rats with ointment quantities that provided nitroglycerin doses of 20, 40 or 80 mg/kg/day from gestation day 7 through gestation day 17 did not result in higher than control incidences of fetal external, visceral or skeletal malformations or variations. Similarly, daily topical application of nitroglycerin ointment to the skin of pregnant rabbits from gestation day 6 through gestation day 18 with ointment quantities that provided nitroglycerin doses up to 80 mg/kg/day in one study and up to 240 mg/kg/day in a second study did not result in higher than control incidences of fetal external, visceral or skeletal malformations or variations.

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Under PRECAUTIONS, Pregnancy:

Pregnancy Category C: Teratology studies in rats and rabbits were conducted with topically applied nitroglycerin ointment at doses of nitroglycerin up to 80 mg nitroglycerin/kg/day and 240 mg nitroglycerin/kg/day, respectively. No toxic effects on dams or fetuses were observed. In a three-generation reproduction study in rats receiving dietary nitroglycerin at doses up to about 434 mg/kg/day there was no evidence of teratogenicity.

There are no adequate and well-controlled studies in pregnant women. CELLEGESIC should be given to a pregnant woman only if clearly needed.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Nitroglycerin (NTG) is effective in relaxing anorectal smooth muscle tissue in experimental animals. Relaxation of anorectal tissue appears to be mediated via the production of nitric oxide, a product of nitroglycerin metabolism, that results in increased intracellular cGMP, leading to smooth muscle relaxation. The sponsor provides a recently published report in which NTG was shown to produce dose-related reductions in the frequency and amplitude of anorectal pressure waves when administered intrarectally in rats as consecutive bolus doses or as a continuous infusion. In addition, the inhibition of anorectal contractions by NTG in these rats occurred without apparent tolerance development.

Following oral administration to rats and dogs, NTG undergoes extensive first-pass metabolism. The mean bioavailability from single oral doses of 3.5 or 7 mg/kg in rats was 1.8% and 1.6%, respectively. In dogs, the bioavailability for oral NTG (0.25 mg/kg) was less than 1%. Topically applied NTG appears to limit first-pass metabolism and yield improved bioavailability. Rectal administration to rats resulted in apparent bioavailability of 27% to 84%. The primary route of biotransformation of NTG in rats is through denitration, yielding glyceryl dinitrate and glyceryl mononitrate metabolites and inorganic nitrite. Most of the products of NTG metabolism following oral administration to rats have been detected in urine and expired air. The urinary metabolites consisted of glyceryl dinitrates, glyceryl mononitrate, glycerol and other polar metabolites of the denitrated products.

Single intravenous or oral doses of NTG administered to mice (6.3 to 25.1 mg/kg IV, 676 to 2408 mg/kg PO) and rats (12.6 to 25.1 mg/kg IV, 539 to 1752 mg/kg PO) resulted in cyanosis, ataxia, respiratory depression and death. Lethal effects were seen within 5 min after IV dosing and within 1 hour after oral dosing.

Dietary administration of NTG for up to 13 weeks in mice (0.5% in diet, 510 mg NTG/kg/day in males and 610 mg NTG/kg/day in females) resulted in no toxicologically significant findings. Dietary administration of NTG (0.01%, 0.1% or 1%) daily to mice for 2 years resulted in pigment deposits (resembling hemosiderin) in the liver, spleen and kidneys of high and mid dose males and females. Methemoglobinemia was observed only in high dose males. The NOAEL in the 2-year mouse study was 96 mg NTG/kg/day in males and 115 mg NTG/kg/day in females. No treatment-related increased incidence of tumors was observed in the 2-year mouse study.

Dietary administration of NTG to rats for up to 13 weeks (0.5% in diet; 230 mg NTG/kg/day in males and females) was associated with decrements in body weight gain and food consumption.

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A dietary dose of 2.5% (~ 1410 mg NTG/kg/day) resulted in lower than control body weight gain and food consumption and higher than control hematopoiesis. Histological findings from the 2.5% dietary dose included pigment deposits in the liver and spleen in males and females and testicular degeneration and/or atrophy in males. Dietary administration of NTG (0.01%, 0.1% or 1%) to rats for 2 years resulted in decrements in body weight gain and food consumption in high dose males and females. Methemoglobinemia (10% to 30% of total hemoglobin) and/or elevated serum enzymes (SGOT, SGPT and alkaline phosphatase) were observed in most high dose males and females dying before scheduled sacrifice. Histopathologic findings included proliferation of bile ducts (high dose males and females) and a dose-related increased incidence and severity of hepatocellular lesions leading to a progressive development of hepatocellular carcinoma. Hepatocellular carcinoma occurred in 13 of 21 high dose males and 11 of 25 high dose females (62% incidence in males and 44% incidence in females; 52% combined incidence vs. 0% for control). A higher than control incidence of neoplastic nodules of the liver was observed in middose males and high dose males and females. A higher than control incidence (11 of 21 vs. 2 of 24) of testicular interstitial cell tumors was noted in high dose males. The NOAEL in rats given NTG for 2 years was 3 and 4 mg/kg/day in males and females, respectively.

Oral doses up to 25 mg NTG/kg/day for 12 months in dogs resulted in an occasional low level of methemoglobinemia. The NOAEL in dogs given NTG for 12 months was 25 mg/kg/day.

NTG tested positive for mutagenicity in the Bacterial Mutagen (Ames) Test and negative for clastogenicity in rat bone marrow cells and in dog lymphocytes *in vivo*. NTG was not mutagenic *in vitro* in aChinese Hamster Ovary assay which was conducted only in the absence of metabolic activation. NTG tested negative in the dominant lethal mutation test.

Dietary administration of NTG for two years (up to 1%; 1022 mg NTG/kg/day in males and 1058 mg NTG/kg/day in females) was not tumorigenic in mice.

A study in rats to explore the mechanism of NTG associated hepatic carcinogenesis revealed K-*ras* point mutations in some of the hepatic tumors. These mutations have also been seen with nitric oxide-induced DNA damage in bacteria, suggesting a role for nitric oxide generation in NTG induced carcinogenesis.

NTG administration to rats in a 3-generation reproduction study revealed adverse effects in the high dose rats (1% NTG in the diet; average NTG intake was 363 mg/kg/day for males and 434 mg/kg/day for females) that included lower than control food intake and consequent poor nutritional status of females, and decreased spermatogenesis in males. Except for a higher than control incidence of incomplete ossification of the hyoid bone and a statistically non-significantly higher than control incidence of diaphragmatic hernia among high dose fetuses, there was no evidence of an NTG-related teratologic effect.

Daily topical application of NTG ointment to the skin of pregnant rats with ointment quantities that provided NTG doses of 20, 40 or 80 mg/kg/day from gestation day 7 through gestation day 17 did not result in higher than control incidences of external, visceral or skeletal fetal malformations or variations. Similarly, daily topical application of NTG ointment to the skin of pregnant rabbits from gestation day 6 through gestation day 18 with ointment quantities that provided NTG doses up to 80 mg/kg/day in one study and up to 240 mg/kg/day in a second study did not result in higher than control incidences of external, visceral or skeletal fetal malformations or variations.

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In a dermal irritation study in rabbits, a lactose-peanut oil paste containing 7.29% NTG was judged to be a mild to moderate irritant. In a dermal sensitivity test in guinea pigs, NTG (3.41% in peanut oil) was shown to be a moderate sensitizing agent

B. Nonclinical Safety Issues Relevant to Clinical Use

This New Drug Application for CELLEGESIC ointment was submitted under the provisions covered by section 505 (b)(2) of the Federal Food, Drug and Cosmetic Act, which permits the sponsor to rely on the Agency's previous findings of safety and/or effectiveness for a drug. Nitroglycerin, the active ingredient in CELLEGESIC ointment, has been used for over a century for the symptomatic relief of angina pectoris. In view of the long-standing clinical use of nitroglycerin, the degree of risk to humans from nitroglycerin based on non-clinical toxicological findings (mutagenesis in one bacterial strain, hepatocellular carcinoma and testicular tumors in rats and aspermatogenesis in rats) is difficult to ascertain. Assessment of carcinogenic potential in rats was conducted with dietary administration of nitroglycerin in which the drug encounters near-complete hepatic first-pass metabolism. Nitroglycerin by dietary administration was not tumorigenic in mice. Assessment of carcinogenicity potential in rodents from topical nitroglycerin administration has not been conducted. Although nitroglycerin tested positive in one bacterial strain in the Ames test, the drug tested negative *in vivo* for mutagenicity (dominant lethal assay in male rats) and clastogenicity (rat bone marrow and dog lymphocyte cytogenetic assays). This suggests that genetic repair mechanisms *in vivo* may blunt the potential mutagenic effects of nitroglycerin. The effectiveness and apparent safety established by the historical use of nitroglycerin has been used to permit the marketing of other nitroglycerin containing formulations, such as the transdermal patch and sublingual spray. Further, the nitroglycerin concentrations (0.2% and 0.4%) in CELLEGESIC ointment are considerably less than that (2%) of the approved topical nitroglycerin ointment formulation, thereby, presumably providing a lower amount of systemic exposure.

Administrative

A. Reviewer signature: _____
Anthony G. Proakis, Ph.D.

B. Supervisor signature: Concurrence - _____
Charles A. Resnick, Ph.D.

C. cc: list:
Orig. NDA # 21,359
HFD-110
HFD-110/Proj. Mgr.
HFD-110/AProakis
HFD-110/CResnick

Reference ID: 2969428

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY

Note: The Pharmacology information described in this section was excerpted from the sponsor's Summary of Nonclinical Pharmacology of nitroglycerin or from referenced published literature.

Primary Pharmacodynamics

Nitroglycerin (NTG), the active ingredient in this product, relaxes most smooth muscle, including bronchial, gastrointestinal, urethral, uterine and vascular. NTG has been widely used for the treatment of angina pectoris and also provides symptomatic relief in congestive heart failure (CHF). The principal pharmacologic action of NTG responsible for its antianginal effect and benefit in CHF is relaxation of vascular smooth muscle and consequent dilatation of peripheral arteries and veins; dilatation of coronary arteries also occurs.

Mechanism of Action

The pharmacological actions of NTG are mediated through the generation of nitric oxide (NO), a signaling molecule produced from metabolism of NTG, that results in increased intracellular cyclic guanosine monophosphate (cGMP) concentrations. NO is also produced endogenously from the amino acid L-arginine by the action of nitric acid synthase within vascular endothelium. NO (from endogenous or NTG-induced sources) diffuses readily from vascular endothelium to the underlying vascular smooth muscle where it activates guanylyl cyclase, a cGMP-dependent protein kinase, to increase intracellular levels of cGMP which results in smooth muscle relaxation.

Drug Activity Related to Proposed Indication

The sponsor provides published reports on the actions of nitroglycerin and nitric oxide on anal-rectal tissues.

Nitric Oxide Pathway in Rectoanal Inhibitory Reflex of Opossum Internal Anal Sphincter, S Rattan, A Sarkar and S Chakder: GASTROENTEROLOGY, vol. 102, 43-50 (1992).

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Release of Nitric Oxide by Activation of Nonadrenergic, Noncholinergic Neurons of Internal Anal Sphincter, S Chakder and S Rattan: AM. J. PHYSIOL., vol 264, G7-G12 (1993).

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Nitroglycerin-Induced Relaxation of Anorectal Smooth Muscle: Evidence for Apparent Lack of Tolerance Development in the Anesthetized Rat, EQ Wang, DM Soda and H Fung: BR. JOURNAL PHARMACOL., vol.134, 418-424 (2001).

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Pharmacology Summary

NTG, the active ingredient in ANOGESIC, relaxes most smooth muscle, including bronchial, gastrointestinal, urethral, uterine and vascular. The pharmacological actions of NTG are mediated through the generation of nitric oxide (NO) that results in increased intracellular cGMP concentrations, leading to smooth muscle relaxation.

Relaxation of isolated smooth muscle strips of opossum internal anal sphincter by electrical field stimulation was accompanied by release of nitric oxide. Also, the NO synthase inhibitor, L-N-nitro-arginine, was shown to cause a dose-dependent suppression of internal anal sphincter relaxation in the anesthetized opossum indicating that nitric oxide is an important of anal sphincter relaxation.

NTG administered intrarectally in rats as consecutive bolus doses or as a continuous infusion produced dose-related reduction in the frequency and amplitude of anorectal pressure waves. The magnitude of anorectal pressure relaxation induced by a bolus dose of NTG was unaffected by prior NTG infusion, indicating that NTG inhibits anorectal contractions in the anesthetized rat without apparent tolerance development.

Pharmacology Conclusions

NTG is effective in producing relaxation of anorectal smooth muscle tissue in experimental animals. Relaxation of anorectal tissue by NTG appears to be mediated via the production of NO that results in increased intracellular cGMP, leading to smooth muscle relaxation. Inhibition of anorectal contractions by NTG in the anesthetized rat occurs without apparent tolerance development.

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II. SAFETY PHARMACOLOGY

No safety pharmacology studies were submitted or cited by the sponsor.

III. PHARMACOKINETICS/TOXICOKINETICS

Note: The Pharmacokinetics and Drug Disposition information described in this section was excerpted from the sponsor's Summary of Non-Clinical Pharmacokinetics of nitroglycerin or from referenced published literature.

Absorption and Pharmacokinetics

Single Dose Studies

Male Sprague Dawley rats were administered single doses of nitroglycerin intracardially at 0.7 mg/kg (n=6), orally at 7 mg/kg (n=5) and topically at 7 mg/kg (n=4) and 14 mg/kg (n=2). The mean half-life after intracardial administration was about 4 min and followed a one-compartment kinetics; the volume of distribution was approximately 3 liters/kg (Table 1). After oral administration, the mean oral bioavailability was determined to be 1.6% (evidence supporting extensive metabolism rather than poor absorption is presented in Metabolism section). No detectable levels of nitroglycerin were observed after topical administration.

Table 1.

Pharmacokinetic Parameters After Intracardiac and Oral Administration in the Rat

	Pharmacokinetic Parameter				
	C_0 (ng/mL)	K_e (min-1)	$t_{½}$ (min)	V_d (L/kg)	$AUC_{0-\infty}$
Intracardial					
Mean	257	0.19	4.2	3.1	1646
SD	125	0.07	1.5	0.9	629
Oral					
Mean	-	0.041	21.4	2.7	265
SD	-	0.018	13.1	0.3	205

The bioavailability of nitroglycerin after rectal administration was investigated in male Sprague Dawley rats. Rectal dosing of nitroglycerin was performed with 3 different lengths of rectal exposure. The unrestricted rectal installation of nitroglycerin solution (3.5 mg/kg of 1.22 mg/ml in normal saline solution) did not lead to complete avoidance of first-pass metabolism (Table 2). When rectal exposure to nitroglycerin was restricted to 3.5 cm or 2.0 cm of lower rectal surface, mean bioavailability increased, but the variability was extremely large. The plasma concentrations obtained > 5 min after rectal exposure were similar to those achieved after IV dosing. These results indicate that that substantial first-pass metabolism of nitroglycerin may be avoided when nitroglycerin is topically applied to the lower rectum.

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Table 2.

Bioavailability of Nitroglycerin (NTG) Following Different Route of Administration

Route of Administration	Dose (mg/kg)	Number of Animals	Peak Plasma Concentration[a] (ng/mL)	AUC[b] (ng/min/mL)	Apparent Bioavailability (%)
Oral	3.5	5	9±5*	0.09±0.04*	1.8±0.9**
Unrestricted rectal	3.5	6	170±58	1.28±0.34	26.7±7.0+
Restricted rectal 3.5 cm	3.5	14	189±181	4.02±3.57	83.8±74.5
Restricted rectal 2.0 cm	1.75	6	129±56	2.19±0.73	91.2± 0.4

[a] Mean ± SD

[b] Area under plasma concentration versus time curve.

NOTE: Symbols indicate significant difference from all other groups (*) from 100% and all other groups (**); and from 100% and restricted rectal (2.0 cm) dosing group (+)

In conscious dogs, the bioavailability of oral NTG (0.25 mg/kg) was very low (0.015%). The formation of dinitrate metabolites was rapid and extensive after IV and PO dosing of NTG (Table 3). The pharmacokinetics of the 2 metabolites was found to be very similar. The glyceryl dinitrates exhibited longer half-lives (approx 40-50 min) and lower apparent clearances than observed for NTG.

Table 3

Mean Pharmacokinetic Parameters of NTG After Low i.v (0.025 mg/kg), High i.v (0.25 mg/kg) and Oral (0.25 mg/kg) Doses to Conscious Dogs

Dose	C_{max} ng/mL	T_{max} min	Total AUC ng/min/mL	Terminal $T_{1/2}$ min	CL_{app} mL/min/kg	$V_{app,ss}$ L/kg
Low i.v						
NTG	5.58±3.60*		21.7±12.2	18±11	1440±630	9.87±4.15
1,2-GDN	25.5±10.4	3±2	834±71	38±4		
1,3-GDN	2.82±0.74	3±1	102±18	39±10		
High i.v						
NTG	91.9±88.7*		463±286	70±42	686±317	10.6±5.4
1,2-GDN	150.0±58	4±1	8346±2256	50±5		
1,3-GDN	37.8±17.1	7±4	2550±885	47±7		
Oral						
NTG	1.5±1.84	1±1	5.60±6.67	4.1±2.4		
1,2-GDN	85.4±53.1	28±14	5477±2354	43±5		
1,3-GDN	55.7±27.9	27±15	3432±1082	46±6		

* Sampling time was 1 minute postdose.

After IV administration of 1.92 mg of ^{14}C-NTG to 3 female monkeys, the decline in plasma NTG was biexponential with an initial half-life of 0.8 min and a terminal half-life of 18 min. After topical administration of a 2% NTG ointment (spread over a 50 cm^2 area of skin) plasma concentrations of unchanged NTG were first detectable at 0.25 hr post application. Peak plasma concentrations of NTG occurred 4 to 6 hours post dose and NTG was still detectable at 24 hours post application. The terminal half-life after topical application was 4.3 hours. The absolute bioavailability of topical NTG was 56.5% (Table 4). The mean bioavailability of ^{14}C products was 72.7%, which includes NTG products produced during percutaneous absorption.

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Table 4

Bioavailability of Topical Nitroglycerin[a]

Pharmacokinetic Parameter	Monkey 1	Monkey 2	Monkey 3	Mean Bioavailability[b]
Dose, mg				
Intravenous	1.92	1.92	1.93	
Topical	18.0	19.7	19.4	
Plasma nitroglycerin				
AUC, *ng hr ml*$^{-1}$				
Intravenous	22.35	18.48	16.60	
Topical	127.98	99.75	93.82	
Bioavailability, %	61.1	52.5	56.2	56.6 ± 2.5
Plasma total radioactivity				
AUC, μg hr ml^{-1}				
Intravenous	6.77	6.92	8.22	
Topical	48.65	63.32	54.44	
Bioavailability, %	76.6	89.2	65.9	77.2 ± 6.7
Urinary total radioactivity				
Intravenous, % dose excreted	60.2	46.6	53.6	
Topical, % dose excreted	39.1	32.2	45.1	
Bioavailability, %	65.0	69.1	84.1	72.7 ± 5.8

[a] Determined from the plasma nitroglycerin AUC, plasma carbon-14 AUC, and urinary excretion of carbon-14. [b] Mean ± *SEM*.

Distribution

Following oral administration of a single dose of ^{14}C-NTG (1/10th of the LD_{50}; LD_{50} in males, 1010 mg/kg; LD_{50} in females, 820 mg/kg), approximately 64% to 90% of the administered radiolabelled dose was absorbed within 24 hours. High levels of radioactivity were detected in the GI tract, liver, urine and expired air (Table 5). The amount of radioactivity in the liver was essentially the same at 4 and 24 hours post dose. The radioactivity ratios (tissue/plasma radioactivity) at 4 and 24 hours post dose indicated that radioactivity was retained in most tissues, especially the liver and kidneys. Most of the absorbed radioactivity from oral administration of NTG was excreted in the urine and expired air. The radioactivity recovered in expired air was approximately 25% of the radiolabelled dose.

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Table 5

Distribution and Excretion of Radioactivity in Rats Receiving a Single Dose of ^{14}C-NTG

Tissue	Percent Administered Dose		Tissue/Plasma Radioactivity Ratio[a]	
	4 hours	24 hours	4 hours	24 hours
GI tract and contents	59.5±13.6[b]	3.0±0.2[c]	-	-
Feces	3.1±1.9	6.3±1.0	-	-
Whole blood[d]	2.1±0.6	6.7±0.0	-	-
Expired air	7.5±1.2	25.5±1.5	-	-
Urine	15.6±4.7	9.8±2.6	-	-
Liver	4.6±0.4	4.3±0.9	4.4±2.0	7.8±1.7
Kidneys	0.7±0.1	0.3±0.0	2.4±0.5	2.8±0.3
Brain	0.3±0.1	0.1±0.0	1.1±0.1	1.0±0.1
Lungs	0.2±0.1	0.1±0.0	1.2±0.1	1.7±0.1
Skeletal muscle[e]	9.3±2.6	2.8±0.2	0.6±0.1	0.6±0.1
Carcass[f]	-	5.4±0.6	-	-
Recovery	102.9±10.0	87.3±7.0	-	-

[a] Radioactivity in 1 mL or gm of wet tissue per radioactivity in 1 mL of plasma
[b] Mean ± SE of three rats
[c] Mean ± SE of four rats
[d] Based on 7% of the body weight
[e] Based on 40% of the body weight
[f] Corrected for radioactivity in muscle

Metabolism

The biotransformation of NTG, like other organic nitrates, is the result of reductive hydrolysis catalyzed by the hepatic enzyme glutathione-organic nitrate reductase. One molecule of NTG reacts with two molecules of reduced glutathione to produce 1, 3- or 1, 2-glyceryl dinitrate and inorganic nitrite. After oral administration of NTG to rats, only small amounts of unchanged parent compound were found in the urine. The urinary metabolites consisted of glyceryl dinitrates, glyceryl mononitrate, glycerol and other polar metabolites of the denitrated products (Table 6).

Table 6

Metabolites in Rat Urine 24 Hours After Oral Administration of ^{14}C-NTG

Metabolite	% Administered Dose
NTG	< 0.1
1,2-GDN	0.7±0.4[a]
1,3-GDN	0.4±0.2
MNGs	10.6±1.3
1,2-GDN-glucuronide	10.0±0.7
1,3-GDN-glucuronide	3.5±0.4
MNG-glucuronides	1.5±0.2
Glycerol	6.9±0.8
Other[b]	
Rf = 0.0	4.9±1.5
Rf = 0.6	1.3±0.3

[a] mean ± SE of three rats
[b] unidentified polar components with a-butanol:acetic acid:water solvent system

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In vitro incubation of NTG with rat liver homogenates did not lead to CO_2 formation even though denitration to glyceryl dinitrate and glyceryl mononitrate proceeded rapidly. Although large amounts of radioactivity ($^{14}CO_2$) in the expired air seen after oral ^{14}C-NTG indicated that the glycerol portion of NTG was extensively metabolized, glycerol was not detected in the liver homogenates after incubation with NTG. There did not appear to be an active enzyme system in the liver which was capable of catalyzing the complete denitration of NTG metabolites to glycerol. The site and mechanism of CO_2 formation from NTG remains unknown.

Excretion

Most of products of NTG metabolism following oral administration of NTG were detected in the urine and expired air. The excretion of radioactivity in the urine after orally administered radiolabelled NTG averaged 30% to 50% of the dose; the radioactivity recovered in expired air averaged 20% to 30% of the NTG dose.

Pharmacokinetics Summary

Following oral administration to rats and dogs, NTG undergoes extensive first-pass metabolism. The mean bioavailability from single oral doses of 3.5 or 7 mg/kg in rats was 1.8% and 1.6%, respectively. In dogs, the bioavailability for oral NTG (0.25 mg/kg) was less than 1%. Topically applied NTG appears to limit first-pass metabolism and yield improved bioavailability. Rectal administration to rats resulted in apparent bioavailability of 27% to 84%. The primary route of biotransformation of NTG is through denitration, yielding glyceryl dinitrate and glyceryl mononitrate metabolites and inorganic nitrite. Most of the products of NTG metabolism following oral administration have been detected in the urine and expired air. The urinary metabolites consisted of glyceryl dinitrates, glyceryl mononitrate, glycerol and other polar metabolites of the denitrated products.

IV. GENERAL TOXICOLOGY

The results of single-dose and repeated-dose toxicity studies described in this section are taken from existing published reports. The sponsor conducted no toxicology studies and, therefore, the sponsor is citing the findings from the existing toxicology literature to identify the non-clinical toxicology profile for NTG.

Single Dose Toxicity Studies

Single dose toxicity studies with oral and IV NTG were conducted in mice and rats. Male and female Swiss albino mice and CD rats were dosed with oral NTG or its metabolites,1,2-GDN or 1,3 GDN, dissolved in a lactose/peanut oil vehicle and observed for 14 days after dosing. Mice and rats treated with toxic doses of NTG and GDNs became cyanotic, exhibited ataxia and had respiratory depression within one hour after dosing (Table 7). Death, presumably due to respiratory depression, occurred on the day of dosing for NTG- and 1,2 GDN treated animals and after 3 to 6 days for the 1,3 GDN treated animals (Table 8).

Male and female CF-1 mice and CD(SD)BR rats were administered single IV doses of NTG (dissolved in 5% aqueous ethanol) in a tail vein and observed for a period of 14 days. Mice and rats treated with lethal doses of NTG died within 5 min after dosing (Table 7). Surviving mice were normal within 2 hours and surviving rats were normal within one hour after dosing.

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The LD_{50} values for oral NTG and its dinitrate metabolites in mice and rats are shown in Table 8.

Table 7

Summary of Single-Dose Toxicity Studies for NTG

Species/Strain	Route of Administration	Doses (mg/kg)	No. and Sex per Group	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Reference
Mice Swiss Albino	Oral	676 to 2,408	6 (male) 6 (female)	1198 (male) 1055 (female)	Toxic doses resulted in cyanosis, ataxia and respiratory depression within one hour	Midwest Research Institute
Rats CD	Oral	539 to 1,752	6 (male) 6 (female)	822 (male) 884 (female)	Toxic doses resulted in cyanosis, ataxia and respiratory depression within one hour	Midwest Research Institute
Mice CF-1	Intravenous[a]	6.31 to 25.1	10 (male) 10 (female)	17.3 (male) 18.2 (female)	All deaths occurred within 5 min of dosing. Surviving animals were normal within 2 h of dosing.	Anderson et al
Rats CD	Intravenous[a]	12.6 to 25.1	10M, 10F per group	24.4 (male) 23.2 (female)	All deaths occurred within 5 min of dosing. Surviving animals were normal within 1 h of dosing.	Anderson et al

[a] In 5% aqueous ethanol

Lethal Doses shown are LD_{50} values

References cited; Anderson JA et.al., J.Applied Toxicology 1983; 3:161-165.
Midwest Research Institute Report No.1for US Army Medical Research; Mammalian Toxicity of Munition Compounds, July 22, 1975.

Table 8.

Oral LD_{50} for NTG and its Metabolites in Mice

	Males		Females	
	LD_{50}±SE mg/kg/day	95% CI	LD_{50}±SE mg/kg.day	95% CI
NTG	1198±76	1008,1352	1055±63	895,1178
1,2-GDN	1221±131	737,1631	1440±130	1217,2328
1,3-GDN	676±68	546,851	688±54	582,836

Oral LD_{50} for NTG and its Metabolites in Rats

	Males		Females	
	LD_{50}±SE mg/kg/day	95% CI	LD_{50}±SE mg/kg/day	95% CI
NTG	822±54	700,953	884±61	763,1055
1,2-GDN	1533±150	1063,1852	1423±176	935,1767
1,3-GDN	1751±192	1335,2248	1056±118	873,1367

Midwest Research Institute Report No.1for US Army Medical Research ; Mammalian Toxicity of Munition Compounds, July 22, 1975

Repeated Dose Toxicity Studies

Repeated oral dose toxicity studies with NTG of up to 13 weeks in duration were conducted in mice, rats and dogs (Table 9). In mice and rats, NTG was administered in the diet (*ad libitum*) at

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NTG concentrations of 0.0% (control), 0.005%, 0.05% or 0.5%. The high dose was equivalent to approximately 560 to 610 mg/kg/day (females and males, respectively) in mice and 230 mg/kg/day in rats. Dogs received 0.05, 0.5 or 5 mg/kg/day of oral NTG in capsules.

Table 9.

Summary of Subchronic Repeated Dose Toxicity Studies for NTG

Species/ Strain	Route of Administration	Duration of Dosing	Doses (mg/kg/day)	No. and Sex per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study Reference
Mice CD-1	Oral (*ad libitum* in feed)	13 weeks	No treatment 1.3-6.9 10.9-60.2 95-608	6M, 6F 6M, 6F 6M, 6F 6M, 6F	60.2	Mild to moderate extramedullary hematopoiesis in liver and spleens in high dose group.	Ellis et al
Rats CD	Oral (*ad libitum* in feed)	13 weeks	No treatment 0.8-3.1 6.0-26.5 59-234	6M, 6F 6M, 6F 6M, 6F 6M, 6F	26.5	At the high dose, decreased food intake and somewhat lower weight gain.	Ellis et al
Rats CD	Oral (*ad libitum* in feed)	13 weeks	No treatment 1406-1416	4M, 4F 3M, 3F	–	Feed consumption lower in treated group. Increase in erythrocytes, reticulocytes, hematocrit, hemoglobin, alkaline phosphatase. No methemoglobinemia seen. Pigment deposits in liver and spleen. Moderate to severe testicular degeneration.	Ellis et al
Rats CD	Intravenous (in 5% aqueous ethanol)	2 weeks	No treatment Vehicle 2.5 5 10	10M, 10F 10M, 10F 10M, 10F 10M, 10F 10M, 10F	10	Body weights and food consumption were reduced in both vehicle and NTG-treated groups. Some local reactions at injection site. No other significant clinical or pathological findings.	Anderson et al
Dogs beagles	Oral (capsules)	13 weeks	No treatment 0.01-0.5 0.1-0.5 1-5	4M, 4F 4M, 4F 4M, 4F 4M, 4F	5	No adverse effects noted.	Ellis et al
Dogs beagles	Oral (capsules)	5 days	No treatment 25 50 100 200	2M, 2F 2M, 2F 2M, 2F 2M, 2F 2M, 2F	5	Methemoglobinemia noted at all doses; increased in a dose-dependent manner. Cyanosis at 100 and 200 mg/kg/day. Decreased activity at 200 mg/kg/day.	Ellis et al
Dogs beagles	Intravenous (in 5% aqueous ethanol)	2 weeks	No treatment Vehicle 1 3	2M, 2F 2M, 2F 2M, 2F 2M, 2F	3	Sporadic licking, salivation and soft stools in both vehicle and NTG-treated groups. No significant clinical or laboratory findings.	Anderson et al

a No observed adverse effect level

References cited: Ellis et al; Fund. Appl. Toxicol., 1984; 4: 248-260
Anderson et al; J. Applied Toxicol, 1983; 3: 161-165

In mice there were no toxicologically significant findings in the hematology, clinical chemistry, gross and microscopic pathology evaluations. Mild to moderate extramedullary hematopoiesis was noted in the liver and/or spleens of some of the high dose males and females given NTG.

For rats, lower than control food consumption and lower than control body weight gain were noted for high dose males and females. There were no toxicologically significant findings in the hematology, clinical chemistry and gross and microscopic pathology evaluations. As only minimal effects were seen at these dose levels of NTG, another 13-week study was conducted in rats fed 2.5% NTG in the diet. Lower than control food consumption and weight gain decrements were noted in males and females during the first 8 weeks; thereafter, food consumption increased. The NTG intake increased throughout the study with an overall average of 1406 mg/kg/day for males and 1416 mg/kg/day for females. NTG-treated rats had higher than control erythrocyte and reticulocyte counts, hematocrit, hemoglobin and serum alkaline phosphatase levels and lower than control blood glucose. Methemoglobinemia was not observed. Histopathologic examination revealed pigment deposits in the liver and spleen in treated males and females and moderate to severe testicular degeneration and/or atrophy in treated males.

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No adverse effects were seen in any male or female dog given up to 5 mg NTG /kg/day orally for 13 weeks. A second repeated dose study using higher doses of NTG (25, 50, 100 or 200 mg/kg/day), but a shorter duration of treatment (5 days), was conducted in dogs. Dose-related methemoglobinemia was observed. The methemoglobin levels in dogs given up to 100 mg/kg/day returned to zero within 24 hours of dosing. At the highest dose (200 mg/kg/day), dogs exhibited cyanosis starting at 2 to 3 hours after dosing and did not show rapid recovery (methemoglobinemia still present 24 hours after dosing).

Repeated oral dose toxicity studies of longer duration were conducted in mice (2 yr), rats (2 yr) and dogs (1yr). Mice and rats received NTG (*ad libitum)* in the diet at concentrations of 0.01, 0.1 and 1% (Table 10).

Table 10

Summary of Chronic Repeated Dose Toxicity Studies for NTG

Species/ Strain	Route of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Reference
Mice CD-1	Oral (NTG as a percent of feed provided *ad libitum*)	2 years	No treatment 9.7-11 96-115 1022-1058	58M, 58F 58M, 58F 58M, 58F 58M, 58F	96-115	High dose level resulted in lowered feed intake, reduced weight gain, failure to groom. Methemoglobinemia, Heinz bodies, and pigment deposition in liver, spleen and kidneys were noted.	Ellis et al
Rats CD	Oral (NTG as a percent of feed provided *ad libitum*)	2 years	No treatment 3.0-4.0 31.5-38.1 363-434	38M, 38F 38M, 38F 38M, 38F 38M, 38F	3-4	At the highest dose, feed consumption was reduced and weight gain decreased. Livers were enlarged, with cholangiofibrosis, proliferation of bile ducts and fibrous tissue. Also seen was progressive development of hepatocellular carcinoma with dose, and interstitial cell tumors in one or both testes in high dose males	Ellis et al
Dogs beagles	Oral (NTG as capsules)	1 year	No treatment 1 5 25	6M, 6F 6M, 6F 6M, 6F 6M, 6F	25	The only effect seen was occasional dose-related methemaglobinemia, with concentrations generally less than 3%.	Ellis et al

Reference cited: Ellis et al; Fund. Appl. Toxicol., 1984; 4: 248-260

Based on food consumption, the high dose male and female mice received 1022 and 1058 mg/kg/day of NTG, respectively; the middle dose males and females received 114.6 and 96.4 mg/kg/day, respectively; the low dose males and females received 11.1 and 9.7 mg/kg/day, respectively (doses represent an average of the daily doses achieved over the course of the study). A lack of grooming was observed in the high dose group after a few months. The incidence of unscheduled deaths was not significantly different among groups. An interim sacrifice was performed after 12 months on 4/sex/dose group. High dose male and female mice killed at 12 months showed a compensated anemia; normal erythrocyte count with elevated reticulocyte count; this effect was also accompanied by Heinz bodies. Methemoglobinemia was noted only in males. The only NTG-related lesion was pigment deposits in the liver, with lesser amounts in spleen and kidneys, of high and mid dose male and female mice (incidence/severity dose-related).

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These deposits resembled hemosiderin. Lesions in mice euthanized at 24 months or dying at unscheduled times were essentially similar to those seen at the 12-month interim sacrifice.

Based on food consumption, the high dose male and female rats received 363 and 434 mg/kg/day of NTG, respectively; the middle dose males and females received 31.5 and 38.1 mg/kg/day, respectively, low dose males and females received 3.04 and 3.99 mg/kg/day, respectively (doses represent an average of the daily doses achieved over the course of the study). Lower than control food consumption and body weight gain were observed for high dose males and females. Lack of grooming was observed among the high dose group after a few months. The primary abnormality seen in the hematologic values was methemoglobinemia, typically 10% to 30% of the total hemoglobin at the end of 3 months in high dose males and females. Erythrocyte count, hematocrit and hemoglobin concentration were higher than control, indicating hemoconcentration. By 12 months, the erythrocyte count returned to normal, but moderate methemoglobinemia still persisted. High dose males had elevations of SGOT, SGPT and alkaline phosphatase. Most high dose rats dying before scheduled sacrifice demonstrated methemoglobinemia and/or elevated serum enzymes (SGOT, SGPT and alkaline phosphatase). No abnormal effects were noted in low dose and middle dose rats. Histopathologic findings included proliferation of the bile ducts and fibrous tissue (cholangiofibrosis) and enlarged livers in a majority of high dose male and female rats. There was a dose-related increased incidence and severity (all dose groups) of foci of hepatocellular alteration, indicating a progressive development of hepatocellular carcinoma. Hepatocellular carcinoma occurred in 13 of 21 high dose males and 11 of 25 high dose females (62% incidence in males and 44% incidence in females; 52% combined incidence vs. 0% for control). Higher than control incidences of neoplastic nodules of the liver were observed in middose males and high dose males and females. A higher than control incidence of testicular interstitial cell tumors was noted in high dose maless(11 of 21 vs. 2 of 24). Dose-related increased incidences of excessive hemosiderin-like pigmentation in the spleen and kidneys was observed mainly in middle and high dose males and females.

The only effect observed in dogs administered 1, 5 or 25 mg/kg/day of NTG orally for 12 months was occasional low level (3%) methemoglobinemia; the incidence was dose-related. Body weight, food consumption, hematology and clinical chemistry parameters were unaffected by NTG treatment. No NTG-related gross or microscopic pathology was observed.

General Toxicology Summary

Single intravenous or oral doses of NTG administered to mice (6.3 to 25.1 mg/kg IV, 676 to 2408 mg/kg PO) and rats (12.6 to 25.1 mg/kg IV, 539 to 1752 mg/kg PO) resulted in cyanosis, ataxia, respiratory depression and death. Lethal effects were seen within 5 min after IV dosing and within 1 hour after oral dosing.

Dietary administration of NTG for up to 13 weeks in mice (0.5% in diet, 510 mg NTG/kg/day in males and 610 mg NTG/kg/day in females) resulted in no toxicologically significant findings. Dietary administration of NTG (0.01%, 0.1% or 1%) daily to mice for 2 years resulted in pigment deposits (resembling hemosiderin) in the liver, spleen and kidneys of high and mid dose males and females. Methemoglobinemia was observed only in high dose males. The NOAEL in the 2-year mouse study was 96 mg NTG/kg/day for males and 115 mg NTG/kg/day for females. No treatment-related increased incidence of tumors was observed in the 2-year mouse study.

Dietary administration of NTG (0.5% in diet; 230 mg NTG/kg/day in males and females) to rats for up to 13 weeks was associated with decrements in body weight gain and food consumption. A

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dietary concentration of 2.5% (~ 1410 mg NTG/kg/day) resulted in lower than control body weight gain and food consumption and higher than control hematopoiesis. Histological findings from the 2.5% dietary dose included pigment deposits in the liver and spleen in males and females and testicular degeneration and/or atrophy in males. Dietary administration of NTG (0.01%, 0.1% or 1%) daily to rats for 2 years resulted in decrements in body weight gain and food consumption in high dose males and females. Methemoglobinemia (10% to 30% of total hemoglobin) and/or elevated serum enzymes (SGOT, SGPT and alkaline phosphatase) were observed in most high dose males and females dying before scheduled sacrifice. Histopathologic findings included proliferation of bile ducts (high dose males and females) and a dose-related increased incidence and severity of hepatocellular lesions indicating a progressive development of hepatocellular carcinoma. Hepatocellular carcinoma occurred in 13 of 21 high dose males and 11 of 25 high dose females (62% incidence in males and 44% incidence in females; 52% combined incidence vs. 0% for control). A higher than control incidence of neoplastic nodules of the liver was observed in middose males and high dose males and females. A higher than control incidence of testicular interstitial cell tumors was noted in high dose males (11 of 21 vs. 2 of 24). The NOAEL for rats given NTG for 2 years was 3 and 4 mg/kg/day for males and females, respectively.

Oral doses up to 25 mg NTG/kg/day for 12 months in dogs resulted in an occasional low level of methemoglobinemia. The NOAEL for dogs given NTG for 12 months was 25 mg/kg/day.

V. GENETIC TOXICOLOGY

NTG was assessed for mutagenic activity in a bacterial mutagen (Ames) test using seven *S. typhymurium* tester strains (TA1975, TA102, TA1535, TA1538, TA100, TA100NR and YG1026) (CM Maragos et al; MUT RES 1993; 298:187-195). Only one *S. typhymurium* strain, TA1535, showed a positive mutagenic response to NTG. NTG concentrations ≥ 2.5 ug/plate caused greater than 2-fold increases in the number of revertant colonies in the absence or presence of metabolic activation. NTG did not cause significant increases in the numbers of bacterial revertants for any of the remaining bacterial strains (histidine reversion less than 2 times background in these strains). The authors concluded that the mutagenicity of NTG was manifest only in the repair-deficient strain (TA1535) which is responsive to single base changes.

Blood samples and kidney tissue were obtained from control and treated rats and dogs after 13 weeks of NTG treatment (approx 230 mg/kg/day in rats and 5 mg/kg/day in dogs) for assessment of genotoxicity *in vivo*. Peripheral blood lymphocytes and kidney cells were cultured. The lymphocytes and kidney cultures were activated, arrested, processed, stained and examined for both numerical and morphological chromosome aberrations. NTG treatment caused no significant increase above control frequency of chromosomal aberrations.

Bone marrow cells and kidney cells were obtained from rats at the end of the 2-year toxicity study and cultured. Actively dividing kidney cell cultures, bone marrow cells and phytohemagluttin-stimulated lymphocytes were arrested at metaphase with colchicine treatment and fixed onto slides for examination of chromosome morphological aberrations. NTG treatment (363 mg/kg/day in males and 434 mg/kg/day in females) caused no significant chromosomal aberrations in this study (Midwest Research Institute Report No 2. for US Army Medical Research; Nov. 11, 1977).

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NTG was tested for its ability to induce single gene mutations in Chinese Hamster Ovary (CHO-K1) cells (Midwest Research Institute Report No 2. for US Army Medical Research; Nov. 11, 1977). NTG concentrations of 50 and 145 ug/ml that caused 65% and 99% cytotoxicity, respectively, did not induce cell mutations. This assay was not conducted in the presence of metabolic activation.

A dominant lethal mutation study was conducted using male rats treated with NTG in the diet (0.01, 0.1 or 1% NTG) for 13 weeks with untreated females. NTG treatment had no adverse effects on fertility, preimplantation loss or post implantation loss. NTG treatment did not result in a dominant lethal mutation effect.

Genetic Toxicology Summary

NTG was positive for mutagenicity in the Bacterial Mutagen (Ames) Test and negative for clastogenicity in rats and dogs in cytogenetic *in vivo* assays. NTG was not mutagenic in a Chinese Hamster Ovary *in vitro* mutagenicity assay which was only conducted in the absence of metabolic activation. NTG was negative in the dominant lethal mutation test.

VI. CARCINOGENICITY

Carcinogenicity assessment of oral NTG in rats and mice is described under General Toxicology, Repeated-Dose Toxicity Studies.

The observation that NTG induced hepatocellular carcinoma in rats after prolonged feeding prompted a further study to evaluate the histogenesis and molecular biology of these tumors and the possible role of nitric oxide (NO), a product of NTG metabolism, in their development (S Tamano et al; CARCINOGENESIS 1996; 17:2477-2486): Male F344 rats received a single intragastric dose (1.2 g/kg) of NTG at 6 weeks of age and/or a diet containing 1% NTG from 8 weeks until necropsy (for up to 78 weeks). Some animals were subjected to 2/3 partial hepatectomy at 9 weeks of age. Five sequential sacrifices (14, 32, 52, 78 and 84 weeks of age) were performed. No liver tumors developed in control rats or in rats receiving a single intragastric dose of NTG, even when dosing was followed by partial hepatectomy. Preneoplastic foci, mainly clear cell and mixed cell type, were found from 14 weeks of age in rats receiving NTG in the diet. Focal eosinophilic areas (atypical foci) composed of atypical hepatocytes that often extended into the veins were observed beginning at 52 weeks of age. Some mixed hepatocholangiocellular adenomas and carcinomas arose in eosinophilic lesions. Hepatocellular carcinomas were seen beginning at 78 weeks of age, but only in rats receiving dietary NTG. The incidence of hepatocellular carcinoma in the latter animals was >50%. Most hepatocellular carcinomas were well differentiated. The carcinogenic effect of NTG given in the diet was not affected by prior administration of a large single dose followed by partial hepatectomy. No *p-53* mutations were found in 18 tumors, but K-*ras* point mutations were found in 8/18 tumors, mostly those of cholangiocellular elements. The investigators conclude that while these mutation types have also been commonly seen in bacteria after nitric oxide-related DNA damage, the fact that the tumors arose only on prolonged exposure to a large dose of this agent seems consistent with a more complex mechanism involving genetic and/or epigenetic effects of NTG

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Carcinogenicity Summary

Administration of NTG up to 1% in the diet (1022 mg/kg/day in males and 1058 mg NTG/kg/day in females) for two years was not tumorigenic in mice.

Dietary administration of NTG to rats (0.1% and 1% in diet; 31.5 mg NTG/kg/day and 363 mg NTG/kg/day, respectively, for males and 38.1 mg NTG/kg/day and 434 mg NTG/kg/day, respectively, for females) was associated with hepatocellular carcinomas in males and females (3/26 and 2/28 in mid-dose males and females respectively, 13/21 and 11/25 in high-dose males and females, respectively, vs 0/24 and 0/29 for male and female controls) and an increased incidence of testicular interstitial cell tumors in high dose males and cholangiofibrosis in high dose males and females. The NOAEL for rats given NTG for 2 years was 0.05% in the diet (3.04 mg NTG/kg/day for males and 3.99 mg NTG/kg/day for females).

A study in rats to explore the mechanism of NTG-associated hepatic carcinogenesis revealed K-*ras* point mutations in some of the hepatic tumors. These mutations have also been seen with nitric oxide-induced DNA damage in bacteria, possibly implicating a role for nitric oxide generation as a mechanism for NTG induced carcinogenesis.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

A 3-generation reproduction toxicity study in CD rats was conducted by the Midwest Research Institute under contract to the US Army (Report No.8; Nov. 8, 1978). Parents and offspring of each generation received diets containing 0.01%, 0.1% or 1% NTG (treatment continued through successive F_1 and F_2 generations). For the F_0 generation, 10 males and 20 females from each dose group were mated after receiving the test diets for 6 months. Each male was housed with 2 females from the same dose group for 14 days. The offspring from the first mating (F_{1a}, first litters) were discarded at weaning, The offspring from the second mating (F_{1b}, 20 to 24 of each sex) within the same dosage group were mated for 14 days at 3 months of age. The F_{2a} generation was discarded at weaning. The F_{1b} males and females were mated for a second time to produce the F_{2b} generation. Males and females from the F_{2b} generation were mated twice beginning at 3 months of age according to the same procedure and the study was terminated upon weaning of the F_{3b} pups. Reproductive performance for each parental generation was measured by the mating ratio (# of copulations to # of male/female pairings) and fertility ratio (# males or females with offspring to the # of that sex mated). Litter measurements included litter size, liveborn index (% of total # of pups liveborn), sex ratio, weight of pups at birth, viability index (# of young alive at day 4 surviving to weaning) and weight at weaning. The 3-generation reproduction study showed severely impaired fertility in F_1 and F_2 high dose males. The first mating of the F_1 high dose rats produced only 3 F_{2a}.litters. The second mating of the F_1 generation resulted in less than a 50% incidence of pregnancy. Although 14 pairs of F_{2a} rats were mated twice, there was only one F_3 litter. When F_{2a} high-dose females were mated with control rats, 13 of 14 rats became pregnant and produced the F_3 generation. Microscopic examination of high dose F_2 males revealed severe aspermatogenesis and mild to moderate increased interstitial tissue in the testis. It was then evident that the poor reproductive performance of the F_2 generation was due to an adverse effect on male fertility. This conclusion was strengthened by the observation that the F_2 males had very small testes and that they produced a high incidence of vaginal plugs without sperm. Most parameters of reproductive performance (litter size, live-born index, birth weight viability and weaning weight), were lower than control in the high dose F_1 and F_2 litters. These adverse effects

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of the high dose of NTG appeared to be secondary to the poor nutritional status of the dams. The feed intake of these high dose rats was about 65% of control. For assessment of NTG induced malformations, the females of the F_0 generation were mated for a third time with untreated males. Fetal weight among offspring from treated F_0 females was comparable to control. Examination of the soft tissues of one-half of the fetuses of the F_0 generation disclosed only a single malformation, diaphragmatic hernia, in the high dose group that appeared to be related to NTG treatment (occurred in 4 of 19 litters vs. 0 of 15 for control, not statistically significant). Skeletal examination of the remaining one-half of the fetuses revealed a higher than control incidence of incomplete ossification of the hyoid bone among high dose fetuses. However, the sternabra, centrum and bones of the skull among NTG treated animals were unaffected.

Published reports describe teratology studies that were conducted in rats and rabbits with dermal application of NTG ointment (Teratology Study of Nitroglycerin Ointment upon Dermal Administration in Rats, VM Skutt,et al, SHINYAKY RINSHO Vol. 34, pp 2009-2023, 1985; Teratologic Test of 10% Nitroglycerin Ointment in Rabbits, S Imoto, THE JOURNAL OF TOXICOLOGICAL SCIENCES, Vol. 11, pp 59-70, 1986; Teratology Study of Nitroglycerin Ointment Upon Dermal Administration in Rabbits, LG Miller et al, SHINYAKU RINSHO, Vol. 34, pp 2024-2032, 1985).

Nitroglycerin 2% ointment was applied topically to the shaved backs of pregnant CD rats on days 7 through 17 of pregnancy. The rats were treated with 1, 2 and 4 gm/kg/day of ointment (providing 20, 40 and 80 mg NTG/kg/day; 36/dose group) for 6 hours (control rats received 4 gm/kg/day of non-NTG containing ointment). After 6 hours the excess ointment was removed to avoid oral intake of the product. On day 20 of pregnancy 2/3 of the dams were sacrificed and the remaining dams were allowed to litter and then sacrificed 21 days after giving birth . Fetuses were examined for external, visceral and skeletal abnormalities. Pups were examined, sexed and weighed at birth and evaluated for development of reflex functions. Dermal application of 2% NTG ointment to pregnant rats had no adverse effects on length of gestation, nesting and nursing behavior of the dams or on the appearance and behavior of the pups. No statistically significant differences in the incidence of fetal malformations or variations were observed between control and NTG-treated groups.

Groups of pregnant New Zealand White rabbits were treated topically with 10% NTG or placebo ointment on days 6 through 18 of pregnancy. The amount of ointment applied to the shaved backs of rabbits was equivalent to 0 (untreated), 0 (placebo), 15, 60 and 240 mg NTG/kg/day (14-16/dose group); the application site was covered with polypropylene film to prevent ointment removal and remained on the animals for 20hrs/day. On day 29 the animals were sacrificed and the fetuses were counted, weighed and examined for external, visceral and skeletal abnormalities. Red spots were noted on the skin of placebo and NTG treated dams but not on untreated controls. NTG treatment had no adverse effect on dams. External, visceral and skeletal examination of fetuses revealed no increases above control (placebo or untreated) incidences of malformations or variations. In a second teratology study in pregnant New Zealand White rabbits, groups of rabbits were treated topically with 2% NTG ointment (1, 2 or 4 gm of ointment providing 20, 40 or 80 mg NTG/kg/day) or placebo on days 6 through 18 of pregnancy. A caesarean section was performed on day 29 of pregnancy and fetuses were examined for the presence of developmental anomalies. No treatment related external, visceral or skeletal malformations or variations were observed.

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Reproductive Toxicology Summary

Dietary administration of NTG to rats in a 3-generation reproduction study revealed adverse effects in the high dose rats (1% NTG in the diet; average NTG intake was 363 mg/kg/day for males and 434 mg/kg/day for females) that included lower than control food intake and consequent poor nutritional status of females, and decreased spermatogenesis and decreased fertility in treated F_1 and F_2 males. Except for the higher than control incidence of incomplete ossification of the hyoid bone and statistically non-significantly higher than control incidence of diaphragmatic hernia among high dose fetuses, there was no other evidence of an NTG-related teratologic effect.

Daily topical application of NTG ointment to the skin of pregnant rats with ointment quantities that provided NTG doses of 20, 40 or 80 mg/kg/day from gestation day 7 through gestation day 17 did not result in higher than control incidences of fetal (external, visceral or skeletal) malformations or variations. Similarly, daily topical application of NTG ointment to the skin of pregnant rabbits from gestation day 6 through gestation day 18 with ointment quantities that provided NTG doses up to 80 mg/kg/day in one study and up to 240 mg/kg/day in a second study did not result in higher than control incidences of fetal (external, visceral or skeletal) malformations or variations.

VIII. SPECIAL TOXICOLOGY STUDIES

Topical Irritation Studies

A primary irritation test and a dermal sensitivity test were performed by the Midwest Research Institute (Report #1, July 22, 1975) for the US Army. New Zealand White rabbits were clipped free of hair on the skin at least 24 hours prior to testing. NTG was prepared as a paste containing 25% peanut oil and 75% of a mixture of NTG in lactose. The paste was calculated to contain 7.29% NTG and 67.21% lactose. A paste comprised of 67% of lactose in peanut oil was used as the vehicle control. After application of the test material, the irritation score on intact and abraded skin was evaluated at 24 and 72 hours. The scoring system used to assess dermal irritation was:

Score Over Controls	Grade of Irritant
> 0.2	mild irritant
> 2.5	moderate irritant
> 5	severe irritant

Based on the irritation score, The NTG test paste was judged to be between a mild to moderate irritant on rabbit skin (Table 11). The primary dinitrate metabolites of NTG were similarly tested and found to be non-irritating in this animal model.

Table 11

Primary Irritation to NTG in Rabbits

Compound	Primary Irritation Score
NTG	0.46
1,2-GDN	< 0.2
1,3-GDN	< 0.2

A dermal sensitivity test was conducted in guinea pigs by prior topical skin patch application of NTG suspension (3.41% NTG in peanut oil) followed by a challenge of topically applied NTG

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suspension 24 hours later. NTG caused a moderate sensitization in 40% (4/10) of the guinea pigs tested indicating that NTG was a moderate sensitizing agent.

Two topical irritation studies conducted for the sponsor by (b) (4) assessed the potential irritation of CELLEGESIC test vehicle formulations (Table 12) on skin and anorectal tissue of New Zealand White rabbits; the formulations tested did not contain NTG.

Table 12

Vehicle Formulations Evaluated in Primary Irritation Studies

	Vehicle Formulation (% w/w)			
Ingredient	DNG 98-001	DNG 98-002	DNG 98-003	DNG 98-004
White Petrolatum USP/NF	66	70	76	100
Paraffin Wax USP/NF	0	4	4	0
Lanolin USP/NF	14	14	14	0
Lactose monohydrate USP/NF	20	0	0	0
Sorbitan sesquioleate NF	0	1	2	0
Propylene glycol USP	0	2	4	0
Total	100	100	100	100

Three test vehicle formulations (65 mg dose of ointments DNG 98-001, DNG 98-002 or DNG 98-003) and one control formulation (DNG 98-004, white petrolatum only) were applied intra-anally (3 rabbits/formulation) to a depth of 1 cm for 5 consecutive days and the perineum was examined for signs of erythema, irritation or discharge. The anorectal tissue from each animal underwent macroscopic and histopathologic examination. No significant differences were observed on gross examination of test and control animals. Histopathologic assessment revealed that all formulations had minimal potential for irritation.

The three test formulations were also applied to the shaved skin of New Zealand White rabbits. The test material (10 uL/cm^2) was applied twice a day (6 to 8 hrs apart) for 7 days. The site of application was examined and scored for evidence of erythema and edema 24 hours after the first application and at 24, 48 and 72 hours after the 3rd and 7th application (values assigned for degree of irritation or edema; none, minimal, mild, moderate or severe, scoring scale not described). Two formulations (DNG 98-001 and DNG 98-002 were determined to be mildly irritating (Irritation Score of 0.1 and 0.3, respectively), whereas the third formulation (DNG 98-003) was determined to be non-irritating (Irritation Score of 0.0).

Special Toxicology Summary

In a dermal irritation study in rabbits, a lactose-peanut oil paste containing 7.29% NTG was judged to be a mild to moderate irritant to rabbit skin. In a dermal sensitivity test in guinea pigs, NTG (3.41% in peanut oil) was shown to be a moderate sensitizing agent

IX. CONCLUSIONS AND RECOMMENDATIONS

This New Drug Application for CELLEGESIC ointment 0.2% and 0.4% (nitroglycerin) was submitted by the sponsor under provisions covered by section 505 (b)(2) of the Federal Food, Drug and Cosmetic Act, which permits the sponsor to rely on the Agency's previous findings of safety and/or effectiveness for a drug. Nitroglycerin, the active ingredient in CELLEGESIC ointment, has been used for over a century for symptomatic relief of angina pectoris; it is

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currently marketed in several dosage forms including sublingual tablets, sublingual spray, oral extended release products, transdermal patch and transdermal ointment. The concentration of nitroglycerin (0.2% and 0.4%) contained in CELLEGESIC ointment and proposed daily doses of NTG in the ointment (1.5 to 4.5 mg/day) are lower than the concentration (2.0%) and recommended daily dose of NTG (6 to 30 mg every 4 to 8 hrs) for a currently marketed transdermal ointment (NITROL®).

The only new information provided in this application by the sponsor is a recently published pharmacology report showing dose-related reductions in the frequency and amplitude of anorectal pressure waves when NTG is administered intrarectally in rats as consecutive bolus doses or as a continuous infusion. The inhibition of anorectal contractions by NTG in these rats occurred without apparent tolerance development. The sponsor also conducted a dermal toxicology study to compare vehicle formulations (without NTG) for skin irritation and sensitivity. The vehicle formulation used in the CELLEGESIC product to be marketed (and used in the clinical trials) is (b) (4) in the animal studies. Other pharmacology, drug disposition, and toxicology information contained in this submission was obtained from published sources.

General Toxicology Issues

The toxicology studies cited by the sponsor and summarized in this review are the same as those (from literature sources) used in assessing the preclinical safety of other nitroglycerin drug products. The sponsor conducted no new toxicology studies other than the dermal irritation and sensitivity studies conducted with their formulation.

Recommendations

This product is approvable from a preclinical perspective. However, statements in the labeling that refer to results of animal toxicity studies should contain the same class-labeling used for other nitroglycerin containing products. Accordingly, the labeling for CELLEGESIC ointment should read as follows:

Under PRECAUTIONS, *Carcinogenesis, Mutagenesis, Impairment of Fertility*:

Rats receiving dietary nitroglycerin for 2 years developed dose-related fibrotic and neoplastic changes in liver (including carcinomas) and interstitial cell tumors in testes. At the highest dose tested (434 mg/kg/day), the incidence of hepatocellular carcinoma for combined males and females was 52% *vs* 0% in controls, and the incidence of testicular tumors was 52% *vs* 8% in controls. Lifetime dietary administration of up to 1058 mg/kg/day of nitroglycerin was not tumorigenic in mice.

Nitroglycerin was weakly mutagenic in Ames tests performed in two different laboratories, but there was no evidence of mutagenicity in an *in vivo* dominant lethal assay with male rats treated with doses up to about 363 mg/kg/day, p.o., or in *in vivo* cytogenetic tests in rat bone marrow cells and in dog lymphocytes. Nitroglycerin was not mutagenic *in vitro* in Chinese Hamster Ovary assay which was conducted only in the absence of metabolic activation.

In a three-generation reproduction study, rats received dietary nitroglycerin for six months prior to mating of the F_0 generation, with treatment continuing through successive F_1 and F_2 generations. The highest dose (434 mg/kg/day) was associated with decreased feed intake and

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body weight gain in both sexes. No specific effect on the fertility of the F_0 generation was seen. Infertility noted in subsequent generations was attributed to the increased interstitial cell tissue and aspermatogenesis in the high-dose males.

Daily topical application of nitroglycerin ointment to the skin of pregnant rats with ointment quantities that provided nitroglycerin doses of 20, 40 or 80 mg/kg/day from gestation day 7 through gestation day 17 did not result in higher than control incidences of fetal external, visceral or skeletal malformations or variations. Similarly, daily topical application of nitroglycerin ointment to the skin of pregnant rabbits from gestation day 6 through gestation day 18 with ointment quantities that provided nitroglycerin doses up to 80 mg/kg/day in one study and up to 240 mg/kg/day in a second study did not result in higher than control incidences of fetal external, visceral or skeletal malformations or variations.

Under the PRECAUTIONS, Pregnancy:

Pregnancy Category C: Teratology studies in rats and rabbits were conducted with topically applied nitroglycerin ointment at doses of nitroglycerin up to 80 mg /kg/day and 240 mg/kg/day, respectively. No toxic effects on dams or fetuses were observed. In a three-generation reproduction study in rats receiving dietary nitroglycerin at doses up to about 434 mg/kg/day there was no evidence of teratogenicity.

There are no adequate and well-controlled studies in pregnant women. CELLEGESIC should be given to a pregnant woman only if clearly needed.

X. APPENDICIES/ATTACHMENTS

None

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

/s/

Anthony Proakis
8/9/04 10:11:48 AM
PHARMACOLOGIST

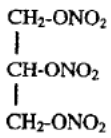
Charles Resnick
8/9/04 10:28:53 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW COVER SHEET

NDA number: 21,359
Review number: 1
Date of submission: 6/22/01 (Sponsor's letter date); 6/26/01 (Center receipt date)
Information to sponsor: Yes ( ) No (X)
Sponsor : Cellegy Pharmaceuticals, Inc.
Manufacturer for drug substance: Nitroglycerin (b) (4) in (b) (4), USP is manufactured by (b) (4)

Reviewer: Anthony G. Proakis, Ph.D.
Division: Cardio-Renal Drug Products
HFD #: 110
Review completion date: 4/10/02
Drug Product: CELLEGESIC Ointment (0.2% and 0.4% w/w nitroglycerin)
Drug:

Generic name: Nitroglycerin
Code name: None
Chemical name: 1,2,3,-propanetriol trinitrate
CAS registry number: 55-63-0
Mole file number:
Molecular formula/molecular weight: $C_3H_5N_3O_9$ / 227.09
Structure:



Relevant IND: Cellegy Pharmaceeuticals IND # 45,326 (IND under which clinical trials were conducted)

Drug Class: Vasodilator

Indication: Relief of pain associated with chronic anal fissure.

Clinical Formulation:

Ingredient	% w/w (0.2%)	% w/w (0.4%)	Function
(b) Nitroglycerin in (b) (4) USP	2.0	4.0	Active Ingredient
White Petrolatum, USP	(b) (4)		
Lanolin, USP			
Propylene Glycol, USP			
Paraffin, NF			
Sorbitan Sesquioleate, NF			

Route of Administration: Applied topically to the anal canal.

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Proposed dosage regimen: A dose of approximately 374 mg of ointment (obtained with a dose-measuring device) is to be applied 2 or 3 times daily until anal pain is gone or the fissure is healed. The amount of NTG contained in the 374 mg dose delivered from the 0.2% ointment is 0.75 mg and from the 0.4% ointment is 1.5% NTG.

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

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Executive Summary

I. Recommendations

A. Recommendation on Approvability

This product is approvable from a non-clinical perspective.

B. Recommendation for Nonclinical Studies

None

C. Recommendations on Labeling

Statements in the labeling that refer to results of animal toxicity studies should be consistent with class-labeling used for other nitroglycerin containing products. Accordingly, the labeling for CELLEGESIC ointment should read as follows:

Under PRECAUTIONS, Carcinogenesis, Mutagenesis, Impairment of Fertility:

Rats receiving dietary nitroglycerin for 2 years developed dose-related fibrotic and neoplastic changes in liver (including carcinomas) and interstitial cell tumors in testes. At the highest dose tested (434 mg/kg/day), the incidence of hepatocellular carcinoma was 62% *vs* 0% in controls for males and 44% *vs* 0% in controls for females, and the incidence of testicular tumors was 52% *vs* 8% in controls. Lifetime dietary administration of up to 1058 mg/kg/day of nitroglycerin was not tumorigenic in mice.

Nitroglycerin was weakly mutagenic in Ames tests performed in two different laboratories, but there was no evidence of mutagenicity in an *in vivo* dominant lethal assay with male rats treated with doses up to about 363 mg/kg/day, p.o., or in *in vivo* cytogenetic tests in rat bone marrow cells and dog lymphocytes. Nitroglycerin was not mutagenic *in vitro* in a Chinese Hamster Ovary assay which was conducted in the absence of metabolic activation.

In a three-generation reproduction study, rats received dietary nitroglycerin for six months prior to mating of the F_0 generation, with treatment continuing through successive F_1 and F_2 generations. The highest dose (434 mg/kg/day) was associated with decreased feed intake and body weight gain in both sexes of all generations. No specific effect on the fertility of the F_0 generation was seen. Infertility noted in subsequent generations was attributed to aspermatogenesis in the high-dose males.

Daily topical application of nitroglycerin ointment to the skin of pregnant rats with ointment quantities that provided nitroglycerin doses of 20, 40 or 80 mg/kg/day from gestation day 7 through gestation day 17 did not result in higher than control incidences of fetal external, visceral or skeletal malformations or variations. Similarly, daily topical application of nitroglycerin ointment to the skin of pregnant rabbits from gestation day 6 through gestation day 18 with ointment quantities that provided nitroglycerin doses up to 80 mg/kg/day in one study and up to 240 mg/kg/day in a second study did not result in higher than control incidences of fetal external, visceral or skeletal malformations or variations.

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Under PRECAUTIONS, Pregnancy:

Pregnancy Category C: Teratology studies in rats and rabbits were conducted with topically applied nitroglycerin ointment at doses of up to 80 mg nitroglycerin/kg/day and 240 mg nitroglycerin/kg/day, respectively. No toxic effects on dams or fetuses were observed. In a three-generation reproduction study in rats receiving dietary nitroglycerin at doses up to about 434 mg/kg/day there was no evidence of teratogenicity.

There are no adequate and well-controlled studies in pregnant women. CELLEGESIC should be given to a pregnant woman only if clearly needed.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Nitroglycerin (NTG) is effective in relaxing anorectal smooth muscle tissue in experimental animals. Relaxation of anorectal tissue appears to be mediated via the production of nitric oxide, a product of nitroglycerin metabolism, that results in increased intracellular cGMP, leading to smooth muscle relaxation. The sponsor provides a recently published report in which NTG was shown to produce dose-related reductions in the frequency and amplitude of anorectal pressure waves when administered intrarectally in rats as consecutive bolus doses or as a continuous infusion. In addition, the inhibition of anorectal contractions by NTG in these rats occurred without apparent tolerance development.

Following oral administration to rats and dogs, NTG undergoes extensive first-pass metabolism. The mean bioavailability from single oral doses of 3.5 or 7 mg/kg in rats was 1.8% and 1.6%, respectively. In dogs, the bioavailability for oral NTG (0.25 mg/kg) was less than 1%. Topically applied NTG appears to limit first-pass metabolism and yield improved bioavailability. Rectal administration to rats resulted in apparent bioavailability of 27% to 84%. The primary route of biotransformation of NTG in rats is through denitration, yielding glyceryl dinitrate and glyceryl mononitrate metabolites and inorganic nitrite. Most of the products of NTG metabolism following oral administration to rats have been detected in urine and expired air. The urinary metabolites consisted of glyceryl dinitrates, glyceryl mononitrate, glycerol and other polar metabolites of the denitrated products.

Single intravenous or oral doses of NTG administered to mice (6.3 to 25.1 mg/kg IV, 676 to 2408 mg/kg PO) and rats (12.6 to 25.1 mg/kg IV, 539 to 1752 mg/kg PO) resulted in cyanosis, ataxia, respiratory depression and death. Lethal effects were seen within 5 min after IV dosing and within 1 hour after oral dosing.

Dietary administration of NTG for up to 13 weeks in mice (0.5% in diet, 510 mg NTG/kg/day in males and 610 mg NTG/kg/day in females) resulted in no toxicologically significant findings. Dietary administration of NTG (0.01%, 0.1% or 1%) daily to mice for 2 years resulted in pigment deposits (resembling hemosiderin) in the liver, spleen and kidneys of high and mid dose males and females. Methemoglobinemia was observed only in high dose males. The NOAEL in the 2-year mouse study was 96 mg NTG/kg/day in males and 115 mg NTG/kg/day in females. No treatment-related increased incidence of tumors was observed in the 2-year mouse study.

Dietary administration of NTG to rats for up to 13 weeks (0.5% in diet; 230 mg NTG/kg/day in males and females) was associated with decrements in body weight gain and food consumption.

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A dietary dose of 2.5% (~ 1410 mg NTG/kg/day) resulted in lower than control body weight gain and food consumption and higher than control hematopoiesis. Histological findings from the 2.5% dietary dose included pigment deposits in the liver and spleen in males and females and testicular degeneration and/or atrophy in males. Dietary administration of NTG (0.01%, 0.1% or 1%) to rats for 2 years resulted in decrements in body weight gain and food consumption in high dose males and females. Methemoglobinemia (10% to 30% of total hemoglobin) and/or elevated serum enzymes (SGOT, SGPT and alkaline phosphatase) were observed in most high dose males and females dying before scheduled sacrifice. Histopathologic findings included proliferation of bile ducts (high dose males and females) and a dose-related increased incidence and severity of hepatocellular lesions leading to a progressive development of hepatocellular carcinoma. Hepatocellular carcinoma occurred in 13 of 21 high dose males and 11 of 25 high dose females (62% incidence in males and 44% incidence in females; 52% combined incidence vs. 0% for control). A higher than control incidence of neoplastic nodules of the liver was observed in middose males and high dose males and females. A higher than control incidence (11 of 21 vs. 2 of 24) of testicular interstitial cell tumors was noted in high dose males. The NOAEL in rats given NTG for 2 years was 3 and 4 mg/kg/day in males and females, respectively.

Oral doses up to 25 mg NTG/kg/day for 12 months in dogs resulted in an occasional low level of methemoglobinemia. The NOAEL in dogs given NTG for 12 months was 25 mg/kg/day.

NTG tested positive for mutagenicity in the Bacterial Mutagen (Ames) Test and negative for clastogenicity in rat bone marrow cells and in dog lymphocytes *in vivo*. NTG was not mutagenic *in vitro* in a Chinese Hamster Ovary assay which was conducted only in the absence of metabolic activation. NTG tested negative in the dominant lethal mutation test.

Dietary administration of NTG for two years (up to 1%; 1022 mg NTG/kg/day in males and 1058 mg NTG/kg/day in females) was not tumorigenic in mice.

A study in rats to explore the mechanism of NTG associated hepatic carcinogenesis revealed K-*ras* point mutations in some of the hepatic tumors. These mutations have also been seen with nitric oxide-induced DNA damage in bacteria, suggesting a role for nitric oxide generation in NTG induced carcinogenesis.

NTG administration to rats in a 3-generation reproduction study revealed adverse effects in the high dose rats (1% NTG in the diet; average NTG intake was 363 mg/kg/day for males and 434 mg/kg/day for females) that included lower than control food intake and consequent poor nutritional status of females, small testes and decreased spermatogenesis in males. Except for a higher than control incidence of incomplete ossification of the hyoid bone and a statistically non-significantly higher than control incidence of diaphragmatic hernia among high dose fetuses, there was no evidence of an NTG-related teratologic effect.

Daily topical application of NTG ointment to the skin of pregnant rats with ointment quantities that provided NTG doses of 20, 40 or 80 mg/kg/day from gestation day 7 through gestation day 17 did not result in higher than control incidences of external, visceral or skeletal fetal malformations or variations. Similarly, daily topical application of NTG ointment to the skin of pregnant rabbits from gestation day 6 through gestation day 18 with ointment quantities that provided NTG doses up to 80 mg/kg/day in one study and up to 240 mg/kg/day in a second study did not result in higher than control incidences of external, visceral or skeletal fetal malformations or variations.

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In a dermal irritation study in rabbits, a lactose-peanut oil paste containing 7.29% NTG was judged to be a mild to moderate irritant. In a dermal sensitivity test in guinea pigs, NTG (3.41% in peanut oil) was shown to be a moderate sensitizing agent

B. Nonclinical Safety Issues Relevant to Clinical Use

This New Drug Application for CELLEGESIC ointment was submitted under the provisions covered by section 505 (b)(2) of the Federal Food, Drug and Cosmetic Act, which permits the sponsor to rely on the Agency's previous findings of safety and/or effectiveness for a drug. Nitroglycerin, the active ingredient in CELLEGESIC ointment, has been used for over a century for the symptomatic relief of angina pectoris. In view of the long-standing clinical use of nitroglycerin, the degree of risk to humans from nitroglycerin based on non-clinical toxicological findings (mutagenesis in one bacterial strain, hepatocellular carcinoma and testicular tumors in rats and aspermatogenesis in rats) is difficult to ascertain. Assessment of carcinogenic potential in rats was conducted with dietary administration of nitroglycerin in which the drug encounters near-complete hepatic first-pass metabolism. Nitroglycerin by dietary administration was not tumorigenic in mice. Assessment of carcinogenicity potential in rodents from topical nitroglycerin administration has not been conducted. Although nitroglycerin tested positive in one bacterial strain in the Ames test, the drug tested negative *in vivo* for mutagenicity (dominant lethal assay in male rats) and clastogenicity (rat bone marrow and dog lymphocyte cytogenetic assays). This suggests that genetic repair mechanisms *in vivo* may blunt the potential mutagenic effects of nitroglycerin. The effectiveness and apparent safety established by the historical use of nitroglycerin has been used to permit the marketing of other nitroglycerin containing formulations, such as the transdermal patch and sublingual spray. Further, the nitroglycerin concentrations (0.2% and 0.4%) in CELLEGESIC ointment are considerably less than that (2%) of the approved topical nitroglycerin ointment formulation, thereby, presumably providing a lower amount of systemic exposure.

Administrative

A. Reviewer signature: _____
Anthony G. Proakis, Ph.D.

B. Supervisor signature: Concurrence - _____
Charles A. Resnick, Ph.D.

C. cc: list:
Orig. NDA # 21,359
HFD-110
HFD-110/Proj. Mgr.
HFD-110/AProakis
HFD-110/CResnick

Reference ID: 2969428

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY

Note: The Pharmacology information described in this section was excerpted from the sponsor's Summary of Nonclinical Pharmacology of nitroglycerin or from referenced published literature.

Primary Pharmacodynamics

Nitroglycerin (NTG), the active ingredient in this product, relaxes most smooth muscle, including bronchial, gastrointestinal, urethral, uterine and vascular. NTG has been widely used for the treatment of angina pectoris and also provides symptomatic relief in congestive heart failure (CHF). The principal pharmacologic action of NTG responsible for its antianginal effect and benefit in CHF is relaxation of vascular smooth muscle and consequent dilatation of peripheral arteries and veins; dilatation of coronary arteries also occurs.

Mechanism of Action

The pharmacological actions of NTG are mediated through the generation of nitric oxide (NO), a signaling molecule produced from metabolism of NTG, that results in increased intracellular cyclic guanosine monophosphate (cGMP) concentrations. NO is also produced endogenously from the amino acid L-arginine by the action of nitric acid synthase within vascular endothelium. NO (from endogenous or NTG-induced sources) diffuses readily from vascular endothelium to the underlying vascular smooth muscle where it activates guanylyl cyclase, a cGMP-dependent protein kinase, to increase intracellular levels of cGMP which results in smooth muscle relaxation.

Drug Activity Related to Proposed Indication

The sponsor provides published reports on the actions of nitroglycerin and nitric oxide on anal-rectal tissues.

Nitric Oxide Pathway in Rectoanal Inhibitory Reflex of Opossum Internal Anal Sphincter, S Rattan, A Sarkar and S Chakder: GASTROENTEROLOGY, vol. 102, 43-50 (1992).

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Release of Nitric Oxide by Activation of Nonadrenergic, Noncholinergic Neurons of Internal Anal Sphincter, S Chakder and S Rattan: AM. J. PHYSIOL., vol 264, G7-G12 (1993).

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Nitroglycerin-Induced Relaxation of Anorectal Smooth Muscle: Evidence for Apparent Lack of Tolerance Development in the Anesthetized Rat, EQ Wang, DM Soda and H Fung: BR. JOURNAL PHARMACOL., vol.134, 418-424 (2001).

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Pharmacology Summary

NTG, the active ingredient in ANOGESIC, relaxes most smooth muscle, including bronchial, gastrointestinal, urethral, uterine and vascular. The pharmacological actions of NTG are mediated through the generation of nitric oxide (NO) that results in increased intracellular cGMP concentrations, leading to smooth muscle relaxation.

Relaxation of isolated smooth muscle strips of opossum internal anal sphincter by electrical field stimulation was accompanied by release of nitric oxide. Also, the NO synthase inhibitor, L-N-nitro-arginine, was shown to cause a dose-dependent suppression of internal anal sphincter relaxation in the anesthetized opossum indicating that nitric oxide is an important of anal sphincter relaxation.

NTG administered intrarectally in rats as consecutive bolus doses or as a continuous infusion produced dose-related reduction in the frequency and amplitude of anorectal pressure waves. The magnitude of anorectal pressure relaxation induced by a bolus dose of NTG was unaffected by prior NTG infusion, indicating that NTG inhibits anorectal contractions in the anesthetized rat without apparent tolerance development.

Pharmacology Conclusions

NTG is effective in producing relaxation of anorectal smooth muscle tissue in experimental animals. Relaxation of anorectal tissue by NTG appears to be mediated via the production of NO that results in increased intracellular cGMP, leading to smooth muscle relaxation. Inhibition of anorectal contractions by NTG in the anesthetized rat occurs without apparent tolerance development.

II. SAFETY PHARMACOLOGY

No safety pharmacology studies were submitted or cited by the sponsor.

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III. PHARMACOKINETICS/TOXICOKINETICS

Note: The Pharmacokinetics and Drug Disposition information described in this section was excerpted from the sponsor's Summary of Non-Clinical Pharmacokinetics of nitroglycerin or from referenced published literature.

Absorption and Pharmacokinetics

Single Dose Studies

Male Sprague Dawley rats were administered single doses of nitroglycerin intracardially at 0.7 mg/kg (n=6), orally at 7 mg/kg (n=5) and topically at 7 mg/kg (n=4) and 14 mg/kg (n=2). The mean half-life after intracardial administration was about 4 min and followed a one-compartment kinetics; the volume of distribution was approximately 3 liters/kg (Table 1). After oral administration, the mean oral bioavailability was determined to be 1.6% (evidence supporting extensive metabolism rather than poor absorption is presented in Metabolism section). No detectable levels of nitroglycerin were observed after topical administration.

Table 1.

Pharmacokinetic Parameters After Intracardiac and Oral Administration in the Rat

	Pharmacokinetic Parameter				
	C_0 (ng/mL)	K_e (min-1)	$t_{½}$ (min)	V_d (L/kg)	$AUC_{0-\infty}$
Intracardial					
Mean	257	0.19	4.2	3.1	1646
SD	125	0.07	1.5	0.9	629
Oral					
Mean	-	0.041	21.4	2.7	265
SD	-	0.018	13.1	0.3	205

The bioavailability of nitroglycerin after rectal administration was investigated in male Sprague Dawley rats. Rectal dosing of nitroglycerin was performed with 3 different lengths of rectal exposure. The unrestricted rectal installation of nitroglycerin solution (3.5 mg/kg of 1.22 mg/ml in normal saline solution) did not lead to complete avoidance of first-pass metabolism (Table 2). When rectal exposure to nitroglycerin was restricted to 3.5 cm or 2.0 cm of lower rectal surface, mean bioavailability increased, but the variability was extremely large. The plasma concentrations obtained > 5 min after rectal exposure were similar to those achieved after IV dosing. These results indicate that that substantial first-pass metabolism of nitroglycerin may be avoided when nitroglycerin is topically applied to the lower rectum.

Table 2.

Bioavailability of Nitroglycerin (NTG) Following Different Route of Administration

Route of Administration	Dose (mg/kg)	Number of Animals	Peak Plasma Concentration[a] (ng/mL)	AUC[b] (ng/min/mL)	Apparent Bioavailability (%)
Oral	3.5	5	9±5*	0.09±0.04*	1.8±0.9**
Unrestricted rectal	3.5	6	170±58	1.28±0.34	26.7±7.0+
Restricted rectal 3.5 cm	3.5	14	189±181	4.02±3.57	83.8±74.5
Restricted rectal 2.0 cm	1.75	6	129±56	2.19±0.73	91.2± 0.4

[a] Mean ± SD
[b] Area under plasma concentration versus time curve.
NOTE: Symbols indicate significant difference from all other groups (*) from 100% and all other groups (**); and from 100% and restricted rectal (2.0 cm) dosing group (+)

In conscious dogs, the bioavailability of oral NTG (0.25 mg/kg) was very low (0.015%). The formation of dinitrate metabolites was rapid and extensive after IV and PO dosing of NTG (Table 3). The pharmacokinetics of the 2 metabolites was found to be very similar. The glyceryl

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dinitrates exhibited longer half-lives (approx 40-50 min) and lower apparent clearances than observed for NTG.

Table 3

Mean Pharmacokinetic Parameters of NTG After Low i.v (0.025 mg/kg), High i.v (0.25 mg/kg) and Oral (0.25 mg/kg) Doses to Conscious Dogs

Dose	C_{max} ng/mL	T_{max} min	Total AUC ng/min/mL	Terminal $T_{1/2}$ min	CL_{app} mL/min/kg	$V_{app,ss}$ L/kg
Low i.v						
NTG	5.58±3.60*		21.7±12.2	18±11	1440±630	9.87±4.15
1,2-GDN	25.5±10.4	3±2	834±71	38±4		
1,3-GDN	2.82±0.74	3±1	102±18	39±10		
High i.v						
NTG	91.9±88.7*		463±286	70±42	686±317	10.6±5.4
1,2-GDN	150.0±58	4±1	8346±2256	50±5		
1,3-GDN	37.8±17.1	7±4	2550±885	47±7		
Oral						
NTG	1.5±1.84	1±1	5.60±6.67	4.1±2.4		
1,2-GDN	85.4±53.1	28±14	5477±2354	43±5		
1,3-GDN	55.7±27.9	27±15	3432±1082	46±6		

* Sampling time was 1 minute posidose.

After IV administration of 1.92 mg of ^{14}C-NTG to 3 female monkeys, the decline in plasma NTG was biexponential with an initial half-life of 0.8 min and a terminal half-life of 18 min. After topical administration of a 2% NTG ointment (spread over a 50 cm^2 area of skin) plasma concentrations of unchanged NTG were first detectable at 0.25 hr post application. Peak plasma concentrations of NTG occurred 4 to 6 hours post dose and NTG was still detectable at 24 hours post application. The terminal half-life after topical application was 4.3 hours. The absolute bioavailability of topical NTG was 56.5% (Table 4). The mean bioavailability of ^{14}C products was 72.7%, which includes NTG products produced during percutaneous absorption.

Table 4

Bioavailability of Topical Nitroglycerin[a]

Pharmacokinetic Parameter	Monkey 1	Monkey 2	Monkey 3	Mean Bioavailability[b]
Dose, mg				
Intravenous	1.92	1.92	1.93	
Topical	18.0	19.7	19.4	
Plasma nitroglycerin				
AUC, *ng hr ml*$^{-1}$				
Intravenous	22.35	18.48	16.60	
Topical	127.98	99.75	93.82	
Bioavailability, %	61.1	52.5	56.2	56.6 ± 2.5
Plasma total radioactivity				
AUC, μg hr ml^{-1}				
Intravenous	6.77	6.92	8.22	
Topical	48.65	63.32	54.44	
Bioavailability, %	76.6	89.2	65.9	77.2 ± 6.7
Urinary total radioactivity				
Intravenous, % dose excreted	60.2	46.6	53.6	
Topical, % dose excreted	39.1	32.2	45.1	
Bioavailability, %	65.0	69.1	84.1	72.7 ± 5.8

[a] Determined from the plasma nitroglycerin AUC, plasma carbon-14 AUC, and urinary excretion of carbon-14. [b] Mean ± *SEM*.

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Distribution

Following oral administration of a single dose of ^{14}C-NTG (1/10th of the LD_{50}; LD_{50} in males, 1010 mg/kg; LD_{50} in females, 820 mg/kg), approximately 64% to 90% of the administered radiolabelled dose was absorbed within 24 hours. High levels of radioactivity were detected in the GI tract, liver, urine and expired air (Table 5). The amount of radioactivity in the liver was essentially the same at 4 and 24 hours post dose. The radioactivity ratios (tissue/plasma radioactivity) at 4 and 24 hours post dose indicated that radioactivity was retained in most tissues, especially the liver and kidneys. Most of the absorbed radioactivity from oral administration of NTG was excreted in the urine and expired air. The radioactivity recovered in expired air was approximately 25% of the radiolabelled dose.

Table 5

Distribution and Excretion of Radioactivity in Rats Receiving a Single Dose of ^{14}C-NTG

	Percent Administered Dose		Tissue/Plasma Radioactivity Ratio[a]	
Tissue	4 hours	24 hours	4 hours	24 hours
GI tract and contents	59.5±13.6[b]	3.0±0.2[c]	-	-
Feces	3.1±1.9	6.3±1.0	-	-
Whole blood[d]	2.1±0.6	6.7±0.0	-	-
Expired air	7.5±1.2	25.5±1.5	-	-
Urine	15.6±4.7	9.8±2.6	-	-
Liver	4.6±0.4	4.3±0.9	4.4±2.0	7.8±1.7
Kidneys	0.7±0.1	0.3±0.0	2.4±0.5	2.8±0.3
Brain	0.3±0.1	0.1±0.0	1.1±0.1	1.0±0.1
Lungs	0.2±0.1	0.1±0.0	1.2±0.1	1.7±0.1
Skeletal muscle[e]	9.3±2.6	2.8±0.2	0.6±0.1	0.6±0.1
Carcass[f]	-	5.4±0.6	-	-
Recovery	102.9±10.0	87.3±7.0	-	-

[a] Radioactivity in 1 mL or gm of wet tissue per radioactivity in 1 mL of plasma
[b] Mean ± SE of three rats
[c] Mean ± SE of four rats
[d] Based on 7% of the body weight
[e] Based on 40% of the body weight
[f] Corrected for radioactivity in muscle

Metabolism

The biotransformation of NTG, like other organic nitrates, is the result of reductive hydrolysis catalyzed by the hepatic enzyme glutathione-organic nitrate reductase. One molecule of NTG reacts with two molecules of reduced glutathione to produce 1, 3- or 1, 2-glyceryl dinitrate and inorganic nitrite. After oral administration of NTG to rats, only small amounts of unchanged parent compound were found in the urine. The urinary metabolites consisted of glyceryl dinitrates, glyceryl mononitrate, glycerol and other polar metabolites of the denitrated products (Table 6).

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Table 6

Metabolites in Rat Urine 24 Hours After Oral Administration of ^{14}C-NTG

Metabolite	% Administered Dose
NTG	< 0.1
1,2-GDN	0.7±0.4[a]
1,3-GDN	0.4±0.2
MNGs	10.6±1.3
1,2-GDN-glucuronide	10.0±0.7
1,3-GDN-glucuronide	3.5±0.4
MNG-glucuronides	1.5±0.2
Glycerol	6.9±0.8
Other[b]	
Rf = 0.0	4.9±1.5
Rf = 0.6	1.3±0.3

[a] mean ± SE of three rats
[b] unidentified polar components with a-butanol:acetic acid:water solvent system

In vitro incubation of NTG with rat liver homogenates did not lead to CO_2 formation even though denitration to glyceryl dinitrate and glyceryl mononitrate proceeded rapidly. Although large amounts of radioactivity ($^{14}CO_2$) in the expired air seen after oral ^{14}C-NTG indicated that the glycerol portion of NTG was extensively metabolized, glycerol was not detected in the liver homogenates after incubation with NTG. There did not appear to be an active enzyme system in the liver which was capable of catalyzing the complete denitration of NTG metabolites to glycerol. The site and mechanism of CO_2 formation from NTG remains unknown.

Excretion

Most of products of NTG metabolism following oral administration of NTG were detected in the urine and expired air. The excretion of radioactivity in the urine after orally administered radiolabelled NTG averaged 30% to 50% of the dose; the radioactivity recovered in expired air averaged 20% to 30% of the NTG dose.

Pharmacokinetics Summary

Following oral administration to rats and dogs, NTG undergoes extensive first-pass metabolism. The mean bioavailability from single oral doses of 3.5 or 7 mg/kg in rats was 1.8% and 1.6%, respectively. In dogs, the bioavailability for oral NTG (0.25 mg/kg) was less than 1%. Topically applied NTG appears to limit first-pass metabolism and yield improved bioavailability. Rectal administration to rats resulted in apparent bioavailability of 27% to 84%. The primary route of biotransformation of NTG is through denitration, yielding glyceryl dinitrate and glyceryl mononitrate metabolites and inorganic nitrite. Most of the products of NTG metabolism following oral administration have been detected in the urine and expired air. The urinary metabolites consisted of glyceryl dinitrates, glyceryl mononitrate, glycerol and other polar metabolites of the denitrated products.

IV. GENERAL TOXICOLOGY

The results of single-dose and repeated-dose toxicity studies described in this section are taken from existing published reports. The sponsor conducted no toxicology studies and, therefore, the

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sponsor is citing the findings from the existing toxicology literature to identify the non-clinical toxicology profile for NTG.

Single Dose Toxicity Studies

Single dose toxicity studies with oral and IV NTG were conducted in mice and rats. Male and female Swiss albino mice and CD rats were dosed with oral NTG or its metabolites,1,2-GDN or 1,3 GDN, dissolved in a lactose/peanut oil vehicle and observed for 14 days after dosing. Mice and rats treated with toxic doses of NTG and GDNs became cyanotic, exhibited ataxia and had respiratory depression within one hour after dosing (Table 7). Death, presumably due to respiratory depression, occurred on the day of dosing for NTG- and 1,2 GDN treated animals and after 3 to 6 days for the 1,3 GDN treated animals (Table 8).

Male and female CF-1 mice and CD(SD)BR rats were administered single IV doses of NTG (dissolved in 5% aqueous ethanol) in a tail vein and observed for a period of 14 days. Mice and rats treated with lethal doses of NTG died within 5 min after dosing (Table 7). Surviving mice were normal within 2 hours and surviving rats were normal within one hour after dosing.

The LD_{50} values for oral NTG and its dinitrate metabolites in mice and rats are shown in Table 8.

Table 7

Summary of Single-Dose Toxicity Studies for NTG

Species/Strain	Route of Administration	Doses (mg/kg)	No. and Sex per Group	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Reference
Mice Swiss Albino	Oral	676 to 2,408	6 (male) 6 (female)	1198 (male) 1055 (female)	Toxic doses resulted in cyanosis, ataxia and respiratory depression within one hour	Midwest Research Institute
Rats CD	Oral	539 to 1,752	6 (male) 6 (female)	822 (male) 884 (female)	Toxic doses resulted in cyanosis, ataxia and respiratory depression within one hour	Midwest Research Institute
Mice CF-1	Intravenous[a]	6.31 to 25.1	10 (male) 10 (female)	17.3 (male) 18.2 (female)	All deaths occurred within 5 min of dosing. Surviving animals were normal within 2 h of dosing.	Anderson et al
Rats CD	Intravenous[a]	12.6 to 25.1	10M, 10F per group	24.4 (male) 23.2 (female)	All deaths occurred within 5 min of dosing. Surviving animals were normal within 1 h of dosing.	Anderson et al

[a] In 5% aqueous ethanol

Lethal Doses shown are LD_{50} values

References cited; Anderson JA et.al., J.Applied Toxicology 1983; 3:161-165.
Midwest Research Institute Report No.1for US Army Medical Research; Mammalian Toxicity of Munition Compounds, July 22, 1975.

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Table 8.

Oral LD_{50} for NTG and its Metabolites in Mice

	Males		Females	
	LD_{50}±SE mg/kg/day	95% CI	LD_{50}±SE mg/kg.day	95% CI
NTG	1198±76	1008,1352	1055±63	895,1178
1,2-GDN	1221±131	737,1631	1440±130	1217,2328
1,3-GDN	676±68	546,851	688±54	582,836

Oral LD_{50} for NTG and its Metabolites in Rats

	Males		Females	
	LD_{50}±SE mg/kg/day	95% CI	LD_{50}±SE mg/kg/day	95% CI
NTG	822±54	700,953	884±61	763,1055
1,2-GDN	1533±150	1063,1852	1423±176	935,1767
1,3-GDN	1751±192	1335,2248	1056±118	873,1367

Midwest Research Institute Report No.1for US Army Medical Research ; Mammalian Toxicity of Munition Compounds, July 22, 1975

Repeated Dose Toxicity Studies

Repeated oral dose toxicity studies with NTG of up to 13 weeks in duration were conducted in mice, rats and dogs (Table 9). In mice and rats, NTG was administered in the diet (*ad libitum*) at NTG concentrations of 0.0% (control), 0.005%, 0.05% or 0.5%. The high dose was equivalent to approximately 560 to 610 mg/kg/day (females and males, respectively) in mice and 230 mg/kg/day in rats. Dogs received 0.05, 0.5 or 5 mg/kg/day of oral NTG in capsules.

Table 9.

Summary of Subchronic Repeated Dose Toxicity Studies for NTG

Species/ Strain	Route of Administration	Duration of Dosing	Doses (mg/kg/day)	No. and Sex per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study Reference
Mice CD-1	Oral (*ad libitum* in feed)	13 weeks	No treatment 1.3-6.9 10.9-60.2 95-608	6M, 6F 6M, 6F 6M, 6F 6M, 6F	60.2	Mild to moderate extramedullary hematopoiesis in liver and spleens in high dose group.	Ellis et al
Rats CD	Oral (*ad libitum* in feed)	13 weeks	No treatment 0.8-3.1 6.0-26.5 59-234	6M, 6F 6M, 6F 6M, 6F 6M, 6F	26.5	At the high dose, decreased food intake and somewhat lower weight gain.	Ellis et al
Rats CD	Oral (*ad libitum* in feed)	13 weeks	No treatment 1406-1416	4M, 4F 3M, 3F	-	Feed consumption lower in treated group. Increase in erythrocytes, reticulocytes, hematocrit, hemoglobin, alkaline phosphatase. No methemoglobinemia seen. Pigment deposits in liver and spleen. Moderate to severe testicular degeneration.	Ellis et al
Rats CD	Intravenous (in 5% aqueous ethanol)	2 weeks	No treatment Vehicle 2.5 5 10	10M, 10F 10M, 10F 10M, 10F 10M, 10F 10M, 10F	10	Body weights and food consumption were reduced in both vehicle and NTG-treated groups. Some local reactions at injection site. No other significant clinical or pathological findings.	Anderson et al
Dogs beagles	Oral (capsules)	13 weeks	No treatment 0.01-0.5 0.1-0.5 1-5	4M, 4F 4M, 4F 4M, 4F 4M, 4F	5	No adverse effects noted.	Ellis et al
Dogs beagles	Oral (capsules)	5 days	No treatment 25 50 100 200	2M, 2F 2M, 2F 2M, 2F 2M, 2F 2M, 2F	5	Methemoglobinemia noted at all doses; increased in a dose-dependent manner. Cyanosis at 100 and 200 mg/kg/day. Decreased activity at 200 mg/kg/day.	Ellis et al
Dogs beagles	Intravenous (in 5% aqueous ethanol)	2 weeks	No treatment Vehicle 1 3	2M, 2F 2M, 2F 2M, 2F 2M, 2F	3	Sporadic licking, salivation and soft stools in both vehicle and NTG-treated groups. No significant clinical or laboratory findings.	Anderson et al

a No observed adverse effect level

References cited: Ellis et al; Fund. Appl. Toxicol., 1984; 4: 248-260
Anderson et al; J. Applied Toxicol, 1983; 3: 161-165

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In mice there were no toxicologically significant findings in the hematology, clinical chemistry, gross and microscopic pathology evaluations. Mild to moderate extramedullary hematopoiesis was noted in the liver and/or spleens of some of the high dose males and females given NTG.

For rats, lower than control food consumption and lower than control body weight gain were noted for high dose males and females. There were no toxicologically significant findings in the hematology, clinical chemistry and gross and microscopic pathology evaluations. As only minimal effects were seen at these dose levels of NTG, another 13-week study was conducted in rats fed 2.5% NTG in the diet. Lower than control food consumption and weight gain decrements were noted in males and females during the first 8 weeks; thereafter, food consumption increased. The NTG intake increased throughout the study with an overall average of 1406 mg/kg/day for males and 1416 mg/kg/day for females. NTG-treated rats had higher than control erythrocyte and reticulocyte counts, hematocrit, hemoglobin and serum alkaline phosphatase levels and lower than control blood glucose. Methemoglobinemia was not observed. Histopathologic examination revealed pigment deposits in the liver and spleen in treated males and females and moderate to severe testicular degeneration and/or atrophy in treated males.

No adverse effects were seen in any male or female dog given up to 5 mg NTG /kg/day orally for 13 weeks. A second repeated dose study using higher doses of NTG (25, 50, 100 or 200 mg/kg/day), but a shorter duration of treatment (5 days), was conducted in dogs. Dose-related methemoglobinemia was observed. The methemoglobin levels in dogs given up to 100 mg/kg/day returned to zero within 24 hours of dosing. At the highest dose (200 mg/kg/day), dogs exhibited cyanosis starting at 2 to 3 hours after dosing and did not show rapid recovery (methemoglobinemia still present 24 hours after dosing).

Repeated oral dose toxicity studies of longer duration were conducted in mice (2 yr), rats (2 yr) and dogs (1yr). Mice and rats received NTG (*ad libitum)* in the diet at concentrations of 0.01, 0.1 and 1% (Table 10).

Table 10

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Summary of Chronic Repeated Dose Toxicity Studies for NTG

Species/ Strain	Route of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Reference
Mice CD-1	Oral (NTG as a percent of feed provided *ad libitum*)	2 years	No treatment 9.7-11 96-115 1022-1058	58M, 58F 58M, 58F 58M, 58F 58M, 58F	96-115	High dose level resulted in lowered feed intake, reduced weight gain, failure to groom. Methemoglobinemia, Heinz bodies, and pigment deposition in liver, spleen and kidneys were noted.	Ellis et al
Rats CD	Oral (NTG as a percent of feed provided *ad libitum*)	2 years	No treatment 3.0-4.0 31.5-38.1 363-434	38M, 38F 38M, 38F 38M, 38F 38M, 38F	3-4	At the highest dose, feed consumption was reduced and weight gain decreased. Livers were enlarged, with cholangiofibrosis, proliferation of bile ducts and fibrous tissue. Also seen was progressive development of hepatocellular carcinoma with dose, and interstitial cell tumors in one or both testes in high dose males	Ellis et al
Dogs beagles	Oral (NTG as capsules)	1 year	No treatment 1 5 25	6M, 6F 6M, 6F 6M, 6F 6M, 6F	25	The only effect seen was occasional dose-related methemaglobinemia, with concentrations generally less than 3%.	Ellis et al

Reference cited: Ellis et al; Fund. Appl. Toxicol., 1984; 4: 248-260

Based on food consumption, the high dose male and female mice received 1022 and 1058 mg/kg/day of NTG, respectively; the middle dose males and females received 114.6 and 96.4 mg/kg/day, respectively; the low dose males and females received 11.1 and 9.7 mg/kg/day, respectively (doses represent an average of the daily doses achieved over the course of the study). A lack of grooming was observed in the high dose group after a few months. The incidence of unscheduled deaths was not significantly different among groups. An interim sacrifice was performed after 12 months on 4/sex/dose group. High dose male and female mice killed at 12 months showed a compensated anemia; normal erythrocyte count with elevated reticulocyte count; this effect was also accompanied by Heinz bodies. Methemoglobinemia was noted only in males. The only NTG-related lesion was pigment deposits in the liver, with lesser amounts in spleen and kidneys, of high and mid dose male and female mice (incidence/severity dose-related). These deposits resembled hemosiderin. Lesions in mice euthanized at 24 months or dying at unscheduled times were essentially similar to those seen at the 12-month interim sacrifice.

Based on food consumption, the high dose male and female rats received 363 and 434 mg/kg/day of NTG, respectively; the middle dose males and females received 31.5 and 38.1 mg/kg/day, respectively, low dose males and females received 3.04 and 3.99 mg/kg/day, respectively (doses represent an average of the daily doses achieved over the course of the study). Lower than control food consumption and body weight gain were observed for high dose males and females. Lack of grooming was observed among the high dose group after a few months. The primary abnormality seen in the hematologic values was methemoglobinemia, typically 10% to 30% of the total hemoglobin at the end of 3 months in high dose males and females. Erythrocyte count, hematocrit and hemoglobin concentration were higher than control, indicating hemoconcentration. By 12 months, the erythrocyte count returned to normal, but moderate methemoglobinemia still persisted. High dose males had elevations of SGOT, SGPT and

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alkaline phosphatase. Most high dose rats dying before scheduled sacrifice demonstrated methemoglobinemia and/or elevated serum enzymes (SGOT, SGPT and alkaline phosphatase). No abnormal effects were noted in low dose and middle dose rats. Histopathologic findings included proliferation of the bile ducts and fibrous tissue (cholangiofibrosis) and enlarged livers in a majority of high dose male and female rats. There was a dose-related increased incidence and severity (all dose groups) of foci of hepatocellular alteration, indicating a progressive development of hepatocellular carcinoma. Hepatocellular carcinoma occurred in 13 of 21 high dose males and 11 of 25 high dose females (62% incidence in males and 44% incidence in females; 52% combined incidence vs. 0% for control). Higher than control incidences of neoplastic nodules of the liver were observed in middose males and high dose males and females. A higher than control incidence of testicular interstitial cell tumors was noted in high dose maless(11 of 21 vs. 2 of 24). Dose-related increased incidences of excessive hemosiderin-like pigmentation in the spleen and kidneys was observed mainly in middle and high dose males and females.

The only effect observed in dogs administered 1, 5 or 25 mg/kg/day of NTG orally for 12 months was occasional low level (3%) methemoglobinemia; the incidence was dose-related. Body weight, food consumption, hematology and clinical chemistry parameters were unaffected by NTG treatment. No NTG-related gross or microscopic pathology was observed.

General Toxicology Summary

Single intravenous or oral doses of NTG administered to mice (6.3 to 25.1 mg/kg IV, 676 to 2408 mg/kg PO) and rats (12.6 to 25.1 mg/kg IV, 539 to 1752 mg/kg PO) resulted in cyanosis, ataxia, respiratory depression and death. Lethal effects were seen within 5 min after IV dosing and within 1 hour after oral dosing.

Dietary administration of NTG for up to 13 weeks in mice (0.5% in diet, 510 mg NTG/kg/day in males and 610 mg NTG/kg/day in females) resulted in no toxicologically significant findings. Dietary administration of NTG (0.01%, 0.1% or 1%) daily to mice for 2 years resulted in pigment deposits (resembling hemosiderin) in the liver, spleen and kidneys of high and mid dose males and females. Methemoglobinemia was observed only in high dose males. The NOAEL in the 2-year mouse study was 96 mg NTG/kg/day for males and 115 mg NTG/kg/day for females. No treatment-related increased incidence of tumors was observed in the 2-year mouse study.

Dietary administration of NTG (0.5% in diet; 230 mg NTG/kg/day in males and females) to rats for up to 13 weeks was associated with decrements in body weight gain and food consumption. A dietary concentration of 2.5% (~ 1410 mg NTG/kg/day) resulted in lower than control body weight gain and food consumption and higher than control hematopoiesis. Histological findings from the 2.5% dietary dose included pigment deposits in the liver and spleen in males and females and testicular degeneration and/or atrophy in males. Dietary administration of NTG (0.01%, 0.1% or 1%) daily to rats for 2 years resulted in decrements in body weight gain and food consumption in high dose males and females. Methemoglobinemia (10% to 30% of total hemoglobin) and/or elevated serum enzymes (SGOT, SGPT and alkaline phosphatase) were observed in most high dose males and females dying before scheduled sacrifice. Histopathologic findings included proliferation of bile ducts (high dose males and females) and a dose-related increased incidence and severity of hepatocellular lesions indicating a progressive development of hepatocellular carcinoma. Hepatocellular carcinoma occurred in 13 of 21 high dose males and 11 of 25 high dose females (62% incidence in males and 44% incidence in females; 52% combined incidence vs. 0% for control). A higher than control incidence of neoplastic nodules of the liver was observed in middose males and high dose males and females. A higher than

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control incidence of testicular interstitial cell tumors was noted in high dose males (11 of 21 vs. 2 of 24). The NOAEL for rats given NTG for 2 years was 3 and 4 mg/kg/day for males and females, respectively.

Oral doses up to 25 mg NTG/kg/day for 12 months in dogs resulted in an occasional low level of methemoglobinemia. The NOAEL for dogs given NTG for 12 months was 25 mg/kg/day.

V. GENETIC TOXICOLOGY

NTG was assessed for mutagenic activity in a bacterial mutagen (Ames) test using seven *S. typhymurium* tester strains (TA1975, TA102, TA1535, TA1538, TA100, TA100NR and YG1026) (CM Maragos et al; MUT RES 1993; 298:187-195). Only one *S. typhymurium* strain, TA1535, showed a positive mutagenic response to NTG. NTG concentrations ≥ 2.5 ug/plate caused greater than 2-fold increases in the number of revertant colonies in the absence or presence of metabolic activation. NTG did not cause significant increases in the numbers of bacterial revertants for any of the remaining bacterial strains (histidine reversion less than 2 times background in these strains). The authors concluded that the mutagenicity of NTG was manifest only in the repair-deficient strain (TA1535) which is responsive to single base changes.

Blood samples and kidney tissue were obtained from control and treated rats and dogs after 13 weeks of NTG treatment (approx 230 mg/kg/day in rats and 5 mg/kg/day in dogs) for assessment of genotoxicity *in vivo*. Peripheral blood lymphocytes and kidney cells were cultured. The lymphocytes and kidney cultures were activated, arrested, processed, stained and examined for both numerical and morphological chromosome aberrations. NTG treatment caused no significant increase above control frequency of chromosomal aberrations.

Bone marrow cells and kidney cells were obtained from rats at the end of the 2-year toxicity study and cultured. Actively dividing kidney cell cultures, bone marrow cells and phytohemagluttin-stimulated lymphocytes were arrested at metaphase with colchicine treatment and fixed onto slides for examination of chromosome morphological aberrations. NTG treatment (363 mg/kg/day in males and 434 mg/kg/day in females) caused no significant chromosomal aberrations in this study (Midwest Research Institute Report No 2. for US Army Medical Research; Nov. 11, 1977).

NTG was tested for its ability to induce single gene mutations in Chinese Hamster Ovary (CHO-K1) cells (Midwest Research Institute Report No 2. for US Army Medical Research; Nov. 11, 1977). NTG concentrations of 50 and 145 ug/ml that caused 65% and 99% cytotoxicity, respectively, did not induce cell mutations. This assay was not conducted in the presence of metabolic activation.

A dominant lethal mutation study was conducted using male rats treated with NTG in the diet (0.01, 0.1 or 1% NTG) for 13 weeks with untreated females. NTG treatment had no adverse effects on fertility, preimplantation loss or post implantation loss. NTG treatment did not result in a dominant lethal mutation effect.

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Genetic Toxicology Summary

NTG was positive for mutagenicity in the Bacterial Mutagen (Ames) Test and negative for clastogenicity in rats and dogs in cytogenetic *in vivo* assays. NTG was not mutagenic in a Chinese Hamster Ovary *in vitro* mutagenicity assay which was only conducted in the absence of metabolic activation. NTG was negative in the dominant lethal mutation test.

VI. CARCINOGENICITY

Carcinogenicity assessment of oral NTG in rats and mice is described under General Toxicology, Repeated-Dose Toxicity Studies.

The observation that NTG induced hepatocellular carcinoma in rats after prolonged feeding prompted a further study to evaluate the histogenesis and molecular biology of these tumors and the possible role of nitric oxide (NO), a product of NTG metabolism, in their development (S Tamano et al; CARCINOGENESIS 1996; 17:2477-2486): Male F344 rats received a single intragastric dose (1.2 g/kg) of NTG at 6 weeks of age and/or a diet containing 1% NTG from 8 weeks until necropsy (for up to 78 weeks). Some animals were subjected to 2/3 partial hepatectomy at 9 weeks of age. Five sequential sacrifices (14, 32, 52, 78 and 84 weeks of age) were performed. No liver tumors developed in control rats or in rats receiving a single intragastric dose of NTG, even when dosing was followed by partial hepatectomy. Preneoplastic foci, mainly clear cell and mixed cell type, were found from 14 weeks of age in rats receiving NTG in the diet. Focal eosinophilic areas (atypical foci) composed of atypical hepatocytes that often extended into the veins were observed beginning at 52 weeks of age. Some mixed hepatocholangiocellular adenomas and carcinomas arose in eosinophilic lesions. Hepatocellular carcinomas were seen beginning at 78 weeks of age, but only in rats receiving dietary NTG. The incidence of hepatocellular carcinoma in the latter animals was >50%. Most hepatocellular carcinomas were well differentiated. The carcinogenic effect of NTG given in the diet was not affected by prior administration of a large single dose followed by partial hepatectomy. No *p-53* mutations were found in 18 tumors, but K-*ras* point mutations were found in 8/18 tumors, mostly those of cholangiocellular elements. The investigators conclude that while these mutation types have also been commonly seen in bacteria after nitric oxide-related DNA damage, the fact that the tumors arose only on prolonged exposure to a large dose of this agent seems consistent with a more complex mechanism involving genetic and/or epigenetic effects of NTG

Carcinogenicity Summary

Administration of NTG up to 1% in the diet (1022 mg/kg/day in males and 1058 mg NTG/kg/day in females) for two years was not tumorigenic in mice.

Dietary administration of NTG to rats (0.1% and 1% in diet; 31.5 mg NTG/kg/day and 363 mg NTG/kg/day, respectively, for males and 38.1 mg NTG/kg/day and 434 mg NTG/kg/day, respectively, for females) was associated with hepatocellular carcinomas in males and females (3/26 and 2/28 in mid-dose males and females respectively, 13/21 and 11/25 in high-dose males and females, respectively, vs 0/24 and 0/29 for male and female controls) and an increased incidence of testicular interstitial cell tumors in high dose males and cholangiofibrosis in high dose males and females. The NOAEL for rats given NTG for 2 years was 0.05% in the diet (3.04 mg NTG/kg/day for males and 3.99 mg NTG/kg/day for females).

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A study in rats to explore the mechanism of NTG-associated hepatic carcinogenesis revealed K-*ras* point mutations in some of the hepatic tumors. These mutations have also been seen with nitric oxide-induced DNA damage in bacteria, possibly implicating a role for nitric oxide generation as a mechanism for NTG induced carcinogenesis.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

A 3-generation reproduction toxicity study in CD rats was conducted by the Midwest Research Institute under contract to the US Army (Report No.8; Nov. 8, 1978). Parents and offspring of each generation received diets containing 0.01%, 0.1% or 1% NTG (treatment continued through successive F_1 and F_2 generations). For the F_0 generation, 10 males and 20 females from each dose group were mated after receiving the test diets for 6 months. Each male was housed with 2 females from the same dose group for 14 days. The offspring from the first mating (F_{1a}, first litters) were discarded at weaning, The offspring from the second mating (F_{1b}, 20 to 24 of each sex) within the same dosage group were mated for 14 days at 3 months of age. The F_{2a} generation was discarded at weaning. The F_{1b} males and females were mated for a second time to produce the F_{2b} generation. Males and females from the F_{2b} generation were mated twice beginning at 3 months of age according to the same procedure and the study was terminated upon weaning of the F_{3b} pups. Reproductive performance for each parental generation was measured by the mating ratio (# of copulations to # of male/female pairings) and fertility ratio (# males or females with offspring to the # of that sex mated). Litter measurements included litter size, liveborn index (% of total # of pups liveborn), sex ratio, weight of pups at birth, viability index (# of young alive at day 4 surviving to weaning) and weight at weaning. The 3-generation reproduction study showed severely impaired fertility in F_1 and F_2 high dose males. The first mating of the F_1 high dose rats produced only 3 F_{2a} litters. The second mating of the F_1 generation resulted in less than a 50% incidence of pregnancy. Although 14 pairs of F_{2a} rats were mated twice, there was only one F_3 litter. When F_{2a} high-dose females were mated with control rats, 13 of 14 rats became pregnant and produced the F_3 generation. Microscopic examination of high dose F_2 males revealed severe aspermatogenesis and mild to moderate increased interstitial tissue in the testis. It was then evident that the poor reproductive performance of the F_2 generation was due to an adverse effect on male fertility. This conclusion was strengthened by the observation that the F_2 males had very small testes and that they produced a high incidence of vaginal plugs without sperm. Most parameters of reproductive performance (litter size, live-born index, birth weight viability and weaning weight), were lower than control in the high dose F_1 and F_2 litters. These adverse effects of the high dose of NTG appeared to be secondary to the poor nutritional status of the dams. The feed intake of these high dose rats was about 65% of control. For assessment of NTG induced malformations, the females of the F_0 generation were mated for a third time with untreated males. Fetal weight among offspring from treated F_0 females was comparable to control. Examination of the soft tissues of one-half of the fetuses of the F_0 generation disclosed only a single malformation, diaphragmatic hernia, in the high dose group that appeared to be related to NTG treatment (occurred in 4 of 19 litters vs. 0 of 15 for control, not statistically significant). Skeletal examination of the remaining one-half of the fetuses revealed a higher than control incidence of incomplete ossification of the hyoid bone among high dose fetuses. However, the sternabra, centrum and bones of the skull among NTG treated animals were unaffected.

Published reports describe teratology studies that were conducted in rats and rabbits with dermal application of NTG ointment (Teratology Study of Nitroglycerin Ointment upon Dermal Administration in Rats, VM Skutt,et al, SHINYAKY RINSHO Vol. 34, pp 2009-2023, 1985;

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Teratologic Test of 10% Nitroglycerin Ointment in Rabbits, S Imoto, THE JOURNAL OF TOXICOLOGICAL SCIENCES, Vol. 11, pp 59-70, 1986; Teratology Study of Nitroglycerin Ointment Upon Dermal Administration in Rabbits, LG Miller et al, SHINYAKU RINSHO, Vol. 34, pp 2024-2032, 1985).

Nitroglycerin 2% ointment was applied topically to the shaved backs of pregnant CD rats on days 7 through 17 of pregnancy. The rats were treated with 1, 2 and 4 gm/kg/day of ointment (providing 20, 40 and 80 mg NTG/kg/day; 36/dose group) for 6 hours (control rats received 4 gm/kg/day of non-NTG containing ointment). After 6 hours the excess ointment was removed to avoid oral intake of the product. On day 20 of pregnancy 2/3 of the dams were sacrificed and the remaining dams were allowed to litter and then sacrificed 21 days after giving birth . Fetuses were examined for external, visceral and skeletal abnormalities. Pups were examined, sexed and weighed at birth and evaluated for development of reflex functions. Dermal application of 2% NTG ointment to pregnant rats had no adverse effects on length of gestation, nesting and nursing behavior of the dams or on the appearance and behavior of the pups. No statistically significant differences in the incidence of fetal malformations or variations were observed between control and NTG-treated groups.

Groups of pregnant New Zealand White rabbits were treated topically with 10% NTG or placebo ointment on days 6 through 18 of pregnancy. The amount of ointment applied to the shaved backs of rabbits was equivalent to 0 (untreated), 0 (placebo), 15, 60 and 240 mg NTG/kg/day (14-16/dose group); the application site was covered with polypropylene film to prevent ointment removal and remained on the animals for 20hrs/day. On day 29 the animals were sacrificed and the fetuses were counted, weighed and examined for external, visceral and skeletal abnormalities. Red spots were noted on the skin of placebo and NTG treated dams but not on untreated controls. NTG treatment had no adverse effect on dams. External, visceral and skeletal examination of fetuses revealed no increases above control (placebo or untreated) incidences of malformations or variations. In a second teratology study in pregnant New Zealand White rabbits, groups of rabbits were treated topically with 2% NTG ointment (1, 2 or 4 gm of ointment providing 20, 40 or 80 mg NTG/kg/day) or placebo on days 6 through 18 of pregnancy. A caesarean section was performed on day 29 of pregnancy and fetuses were examined for the presence of developmental anomalies. No treatment related external, visceral or skeletal malformations or variations were observed.

Reproductive Toxicology Summary

Dietary administration of NTG to rats in a 3-generation reproduction study revealed adverse effects in the high dose rats (1% NTG in the diet; average NTG intake was 363 mg/kg/day for males and 434 mg/kg/day for females) that included lower than control food intake and consequent poor nutritional status of females, and decreased spermatogenesis and decreased fertility in treated F_1 and F_2 males. Except for the higher than control incidence of incomplete ossification of the hyoid bone and statistically non-significantly higher than control incidence of diaphragmatic hernia among high dose fetuses, there was no other evidence of an NTG-related teratologic effect.

Daily topical application of NTG ointment to the skin of pregnant rats with ointment quantities that provided NTG doses of 20, 40 or 80 mg/kg/day from gestation day 7 through gestation day 17 did not result in higher than control incidences of fetal (external, visceral or skeletal) malformations or variations. Similarly, daily topical application of NTG ointment to the skin of pregnant rabbits from gestation day 6 through gestation day 18 with ointment quantities that

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provided NTG doses up to 80 mg/kg/day in one study and up to 240 mg/kg/day in a second study did not result in higher than control incidences of fetal (external, visceral or skeletal) malformations or variations.

VIII. SPECIAL TOXICOLOGY STUDIES

Topical Irritation Studies

A primary irritation test and a dermal sensitivity test were performed by the Midwest Research Institute (Report #1, July 22, 1975) for the US Army. New Zealand White rabbits were clipped free of hair on the skin at least 24 hours prior to testing. NTG was prepared as a paste containing 25% peanut oil and 75% of a mixture of NTG in lactose. The paste was calculated to contain 7.29% NTG and 67.21% lactose. A paste comprised of 67% of lactose in peanut oil was used as the vehicle control. After application of the test material, the irritation score on intact and abraded skin was evaluated at 24 and 72 hours. The scoring system used to assess dermal irritation was:

Score Over Controls	Grade of Irritant
> 0.2	mild irritant
> 2.5	moderate irritant
> 5	severe irritant

Based on the irritation score, The NTG test paste was judged to be between a mild to moderate irritant on rabbit skin (Table 11). The primary dinitrate metabolites of NTG were similarly tested and found to be non-irritating in this animal model.

Table 11

Primary Irritation to NTG in Rabbits

Compound	Primary Irritation Score
NTG	0.46
1,2-GDN	< 0.2
1,3-GDN	< 0.2

A dermal sensitivity test was conducted in guinea pigs by prior topical skin patch application of NTG suspension (3.41% NTG in peanut oil) followed by a challenge of topically applied NTG suspension 24 hours later. NTG caused a moderate sensitization in 40% (4/10) of the guinea pigs tested indicating that NTG was a moderate sensitizing agent.

Two topical irritation studies conducted for the sponsor by [redacted] (b) (4) assessed the potential irritation of CELLEGESIC test vehicle formulations (Table 12) on skin and anorectal tissue of New Zealand White rabbits; the formulations tested did not contain NTG.

Table 12

Vehicle Formulations Evaluated in Primary Irritation Studies

	Vehicle Formulation (% w/w)			
Ingredient	DNG 98-001	DNG 98-002	DNG 98-003	DNG 98-004
White Petrolatum USP/NF	66	70	76	100
Paraffin Wax USP/NF	0	4	4	0
Lanolin USP/NF	14	14	14	0
Lactose monohydrate USP/NF	20	0	0	0
Sorbitan sesquioleate NF	0	1	2	0
Propylene glycol USP	0	2	4	0
Total	100	100	100	100

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Three test vehicle formulations (65 mg dose of ointments DNG 98-001, DNG 98-002 or DNG 98-003) and one control formulation (DNG 98-004, white petrolatum only) were applied intra-anally (3 rabbits/formulation) to a depth of 1 cm for 5 consecutive days and the perineum was examined for signs of erythema, irritation or discharge. The anorectal tissue from each animal underwent macroscopic and histopathologic examination. No significant differences were observed on gross examination of test and control animals. Histopathologic assessment revealed that all formulations had minimal potential for irritation.

The three test formulations were also applied to the shaved skin of New Zealand White rabbits. The test material (10 uL/cm^2) was applied twice a day (6 to 8 hrs apart) for 7 days. The site of application was examined and scored for evidence of erythema and edema 24 hours after the first application and at 24, 48 and 72 hours after the 3rd and 7th application (values assigned for degree of irritation or edema; none, minimal, mild, moderate or severe, scoring scale not described). Two formulations (DNG 98-001 and DNG 98-002 were determined to be mildly irritating (Irritation Score of 0.1 and 0.3, respectively), whereas the third formulation (DNG 98-003) was determined to be non-irritating (Irritation Score of 0.0).

Special Toxicology Summary

In a dermal irritation study in rabbits, a lactose-peanut oil paste containing 7.29% NTG was judged to be a mild to moderate irritant to rabbit skin. In a dermal sensitivity test in guinea pigs, NTG (3.41% in peanut oil) was shown to be a moderate sensitizing agent

IX. CONCLUSIONS AND RECOMMENDATIONS

This New Drug Application for CELLEGESIC ointment 0.2% and 0.4% (nitroglycerin) was submitted by the sponsor under provisions covered by section 505 (b)(2) of the Federal Food, Drug and Cosmetic Act, which permits the sponsor to rely on the Agency's previous findings of safety and/or effectiveness for a drug. Nitroglycerin, the active ingredient in CELLEGESIC ointment, has been used for over a century for symptomatic relief of angina pectoris; it is currently marketed in several dosage forms including sublingual tablets, sublingual spray, oral extended release products, transdermal patch and transdermal ointment. The concentration of nitroglycerin (0.2% and 0.4%) contained in CELLEGESIC ointment and proposed daily doses of NTG in the ointment (1.5 to 4.5 mg/day) are lower than the concentration (2.0%) and recommended daily dose of NTG (6 to 30 mg every 4 to 8 hrs) for a currently marketed transdermal ointment (NITROL®).

The only new information provided in this application by the sponsor is a recently published pharmacology report showing dose-related reductions in the frequency and amplitude of anorectal pressure waves when NTG is administered intrarectally in rats as consecutive bolus doses or as a continuous infusion. The inhibition of anorectal contractions by NTG in these rats occurred without apparent tolerance development. The sponsor also conducted a dermal toxicology study to compare vehicle formulations (without NTG) for skin irritation and sensitivity. The vehicle formulation used in the CELLEGESIC product to be marketed (and used in the clinical trials) is (b) (4)

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(b) (4). Other pharmacology, drug disposition, and toxicology information contained in this submission was obtained from published sources.

General Toxicology Issues

The toxicology studies cited by the sponsor and summarized in this review are the same as those (from literature sources) used in assessing the preclinical safety of other nitroglycerin drug products. The sponsor conducted no new toxicology studies other than the dermal irritation and sensitivity studies conducted with their formulation.

Recommendations

This product is approvable from a preclinical perspective. However, statements in the labeling that refer to results of animal toxicity studies should contain the same class-labeling used for other nitroglycerin containing products. Accordingly, the labeling for CELLEGESIC ointment should read as follows:

Under PRECAUTIONS, Carcinogenesis, Mutagenesis, Impairment of Fertility:

Rats receiving dietary nitroglycerin for 2 years developed dose-related fibrotic and neoplastic changes in liver (including carcinomas) and interstitial cell tumors in testes. At the highest dose tested (434 mg/kg/day), the incidence of hepatocellular carcinoma was 62% *vs* 0% in controls for males and 44% *vs* 0% in controls for females, and the incidence of testicular tumors was 52% *vs* 8% in controls. Lifetime dietary administration of up to 1058 mg/kg/day of nitroglycerin was not tumorigenic in mice.

Nitroglycerin was weakly mutagenic in Ames tests performed in two different laboratories, but there was no evidence of mutagenicity in an *in vivo* dominant lethal assay with male rats treated with doses up to about 363 mg/kg/day, p.o., or in *in vivo* cytogenetic tests in rat bone marrow cells and dog lymphocytes. Nitroglycerin was not mutagenic *in vitro* in a Chinese Hamster Ovary assay which was conducted in the absence of metabolic activation.

In a three-generation reproduction study, rats received dietary nitroglycerin for six months prior to mating of the F_0 generation, with treatment continuing through successive F_1 and F_2 generations. The highest dose (434 mg/kg/day) was associated with decreased feed intake and body weight gain in both sexes of all generations. No specific effect on the fertility of the F_0 generation was seen. Infertility noted in subsequent generations was attributed to aspermatogenesis in the high-dose males.

Daily topical application of nitroglycerin ointment to the skin of pregnant rats with ointment quantities that provided nitroglycerin doses of 20, 40 or 80 mg/kg/day from gestation day 7 through gestation day 17 did not result in higher than control incidences of fetal external, visceral or skeletal malformations or variations. Similarly, daily topical application of nitroglycerin ointment to the skin of pregnant rabbits from gestation day 6 through gestation day 18 with ointment quantities that provided nitroglycerin doses up to 80 mg/kg/day in one study and up to 240 mg/kg/day in a second study did not result in higher than control incidences of fetal external, visceral or skeletal malformations or variations.

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Under PRECAUTIONS, Pregnancy:

Pregnancy Category C: Teratology studies in rats and rabbits were conducted with topically applied nitroglycerin ointment at doses of up to 80 mg nitroglycerin/kg/day and 240 mg nitroglycerin/kg/day, respectively. No toxic effects on dams or fetuses were observed. In a three-generation reproduction study in rats receiving dietary nitroglycerin at doses up to about 434 mg/kg/day there was no evidence of teratogenicity.

There are no adequate and well-controlled studies in pregnant women. CELLEGESIC should be given to a pregnant woman only if clearly needed.

X. APPENDICIES/ATTACHMENTS

None

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/s/

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4/10/02 03:13:33 PM
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This is a pharm/tox review of a 505(b)(2) application.