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APPLICATION NUMBER: NDA 21134

PHARMACOLOGY REVIEW(S)

FROM
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NDA 21-134

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NITROSTAT
0.3 mg, 0.4 mg and 0.6 mg
Nitroglycerin Sublingual Tablets

PARKE-DAVIS PHARMACEUTICALS, LIMITED
Vega Baja, Puerto Rico 00694-4119

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REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

NDA 21-134

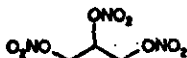
KEY WORDS: Nitroglycerin, Sublingual tablets
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SPONSOR: Parke-Davis Pharmaceutical Research
Division of Warner-Lambert Company
Ann Arbor, Michigan 48105

DRUG

Generic Name: Nitroglycerin (USAN)
Code Numbers: CI-782; PD 79964; DNG
Synonyms: glyceryl trinitrate; trinitroglycerin, glycerol nitric acid triester, glonoin, trinitrin, blasting gelatin; blasting oil, TNG, S.N.G., and others.
Trade Name: Nitrostat
Chemical Name: 1,2,3-propanetriol trinitrate
CAS Registry Number: 55-63-0
Molecular Formula//M. Weight: $C_3H_5N_3O_9$ //227.09
Chemical Structure:



Manufacturer of active substance: {
(DMF.
Manufacturer of nitroglycerin tablets: Parke-Davis
Fajardo, Puerto Rico.
Relevant INDS/DMFs: }
DMFs: []

DRUG CLASS: Smooth muscle relaxant primarily affecting vascular smooth muscle. In humans, nitroglycerin primarily induces venodilation.

THERAPEUTIC INDICATION: Coronary vasodilator.

CLINICAL FORMULATION: Sublingual tablets containing 0.3, 0.4 and 0.6 mg Diluted Nitroglycerin, USP, 1.95%.

Degradation Products: The NDA stated that other degradation products 2-mononitroglycerin (2-MNG); 1-mononitroglycerin (1-MNG) and clonitrate were monitored for in the sublingual tablets made in Vega Baja, Puerto Rico and none were detected. (V.2, p.73-74.)

PREVIOUS CLINICAL EXPERIENCE: Nitroglycerin (NTG) sublingual tablets are marketed in US as coronary vasodilators (antianginal drug). The present formulation is also marketed in Canada, Hong Kong, Korea, Netherlands, Philippines and Taiwan.

Drug Interactions: When patients take nitrate therapy, high levels of nitric oxide (NO) are present in the vascular smooth muscle. Sildenafil (Viagra) potentiates the hypotensive effects of nitrates because this drug inhibits type CGMP phosphodiesterase which is present in vascular smooth muscle cells. The potentiation of the vasodilator effect of circulating NO results in a significant fall in blood pressure (BP). Other drugs interacting with NTG also exaggerate its hypotensive effects.

Regarding drugs interacting with NTG, a journal article provided by drug sponsor (V.1; Ref. 4), FJ DiCarlo* reported that NTG interacts with a variety of compounds. The article stated that the smooth muscle relaxing effect of NTG is antagonized by histamine, norepinephrine/epinephrine and acetylcholine; that NTG potentiates the hypotensive and anticholinergic effects of tricyclic antidepressants, the hypotensive effects of meperidine and the anticholinergic effects of certain anticholinergics and antihistaminic drugs. No nonclinical studies were reported for these drug interactions findings.

INTRODUCTION and DRUG HISTORY: Briefly, some literature reports traced the medical use of organic nitrates back to the XIIIth - XIVth century. Liquid NTG was discovered in 1846 by the Italian chemist Ascanio Sobrero. It was found that upon percussion, liquid NTG explodes with the release of various gases. Sobrero was said to have described a migraine headache while working with the compound. Constantin Hering, a practitioner of homeopathic medicine in the US, read Sobrero's report, obtained NTG and tested the chemical on his students. The results of Hering's studies suggested a potential therapeutic use for NTG in the treatment of several medical conditions.

* FJ DiCarlo in "Nitroglycerin Revisited: Chemistry, Biochemistry, Interactions", Drug Metabolism Reviews V4 (1), 1975

The literature also reports that angina pectoris (the symptomatic manifestation of myocardial ischemia) was first described as a distinct clinical entity in the latter part of the XVIIIth century by William Heberden. By the XIXth century, a sublingual dosage form of NTG had been developed and, the drug was prescribed for the treatment of different conditions (e.g., epilepsy, cardiac edema, and headache).

After the observation by Guthrie in 1859 that inhalation of amyl nitrate rapidly relieved, but only for a short time, anginal pain, William Murrell in 1879 tested NTG in patients with angina pectoris. Thus, some sources credit Murrell with establishing the use of sublingual NTG for relief of acute angina pectoris. Other sources also credit the use of NTG, as a classical coronary vasodilator, to W. Evans, C. Hoyle (1934) and H.K. Russek (1950).

Although undiluted NTG is/may be used in the manufacture of dynamite, when NTG is diluted with inert excipients (e.g., lactose, dextrose, etc), it may be safely handled and formulated into tablets.* However, since this chemical can escape from the tablets, stabilizers are added to decrease its vapor pressure.

NONCLINICAL STUDIES REVIEWED: Although NTG has been used clinically as a coronary vasodilator for many years and nonclinical studies with the drug were submitted in an ANDA (to Division of Generic Drugs- DGD for a transdermal system between 1991-1993 by G.D. Searle & Co), these nonclinical studies were apparently first evaluated by PHARMACOLOGY at DCDRP. The evaluation was done to determine whether the nonclinical data provided supported a proposed "labeling update" for the marketed transdermal system Nitrodisc. (The application for this transdermal system was transferred from DGD to DCDRP in ~ 1993).

For the present NDA (NDA 21-134, Parke-Davis Pharm Inc, NTG sublingual tablet formulation), no new nonclinical studies were submitted and none were required. At the IND level, no nonclinical studies had been conducted, submitted or required Pharmacology and Toxicology Review dated 11-27-85). However, at this the NDA level, published nonclinical studies were submitted in the form of a summary document of old studies: Trinitroglycerol: Health Advisory (HA) dated 09-25-87. This HA had been prepared by the Office of Drinking Water, US Environmental Protection Agency (EPA), using data from NTG studies conducted mainly by () for the US Army under contract) between the years of 1975-1978. These nonclinical studies had been conducted under the direction of different investigators and, none of the studies were reported as complying with GLP regulations.

* NTG is marketed in several delivery systems (i.e., oral formulations as immediate or extended release tablets and sprays, injectables, topical ointments, patches, and discs for transdermal administration).

The following is a brief overview of data extracted from some journal articles submitted by the drug sponsor, the HA document, and from the Review and Evaluation of Toxicology Data: NTG Transdermal System (Nitrodisc): Labeling Update in DCDRP Files.

•> **PHARMACOLOGY:** Limited nonclinical pharmacology data were found in the journal articles submitted by the drug sponsor; studies found were from studies conducted/reported over a decade ago. Most findings reported are consistent with the hypothesis that NTG and other organic nitrates are pro-drugs that exert their pharmacologic activity only after metabolism at their site of action. Other studies reported showed that under physiological conditions, rat serum hydrolyses NTG to dinitrates and mononitrates; the T% of serum degradation was reported to be ~ 20 min at 37°C.

NTG undergoes biotransformation to glyceryl dinitrates prior to in vitro relaxation of rabbit aortic strips, and bovine pulmonary vein and artery. Glyceryl 1-2 dinitrate (1-2, GDN) and glyceryl 1-3 dinitrate (1-3, GDN) are 2 of the reported NTG metabolites.

The in vivo pharmacologic properties of 1-3, GDN and 1-2, GDN were investigated and compared to the parent compound- NTG. In guinea pig, both metabolites were found to lower the blood pressure (BP) in this preparation. $ED_{15 \text{ mm}}$ was determined as the dose necessary to lower the BP by 15 mm in the preparation.

Results reported indicate that the $ED_{15 \text{ mm}}$ values for the 2 metabolites were larger (less potent) than NTG; the BP reducing potency of each of the metabolites did not differ significantly from each other.

In dog perfused hindleg, the two metabolites were less potent than NTG in reducing the mean perfusion pressure. The ED_{25} dose for NTG (the dose needed to reduce the perfusion pressure by 25 mm) was lower than that for the two metabolites; the $ED_{25 \text{ mm}}$ for GTN 1-2 and GTN 1-3 were ~ 28 and ~ 19 X higher than that of NTG. The dose-response curves for these 3 compounds were reported to be parallel. From these limited studies, the investigators concluded the 2 metabolites should not be considered to account for the effect of NTG. In dogs, the dinitrate metabolites were reported to be less active than the parent compound on BP. However, in dogs, after repeated or continuous exposure to NTG, tolerance develops to its hypotensive effect in a dose/time-dependent manner.

Several different mechanism have been proposed to explain the relaxing effect of organic nitrates esters on vascular smooth muscle. The currently proposed mechanism of action of NTG in the relief of an attack or prophylaxis of angina pectoris which appears on the label of Nitrostat tablets states:

"Nitroglycerin forms free radical nitric oxide (NO) which activates guanylate cyclase, resulting in an increase of guanosine 3'5' monophosphate (cyclic GMP) in smooth muscle and other tissues. This eventually leads to dephosphorylation of the light chain of myosin, which regulates the contractile state in smooth muscle, resulting in vasodilation."

•> SAFETY PHARMACOLOGY: None reported.

•> TOXICOLOGY:

Since humans are exposed to NTG in clinical/occupational settings, some US Government agencies anticipated that NTG might become an environmental contaminant. Thus, the previously mentioned HA was developed in 1987 by the EPA to provide information on the health effects, actions and methods of analysis/treatment of the water supply, if contaminated by NTG.

The HA document includes nonclinical studies conducted with NTG by different investigators. As noted above, NTG TOXICOLOGY studies were conducted between 1975-1978 for the US Army and the studies were evaluated in DCRDP for the "labeling update" of a marketed NTG transdermal system. (See results/conclusions of that evaluation in the "Nitroglycerin Transdermal System: (Nitrodisc) Labeling Update: dated 03-29-93.") From this document, pertinent toxicologic findings on NTG were extracted and condensed in the version below.

The table below, prepared by the drug sponsors, lists some studies conducted with NTG. Not included in the list, are genotoxicity assays (in vitro Ames test and cytogenic analysis in mammalian cells, chromosome aberration in CHO-K1 cells and, in vivo dominant lethal test in rat) and an oral 3-generation reproduction studies in rats.

Summary of Toxicity Studies on TNC in Animals

<u>Reference</u>	<u>Species</u>	<u>Dose</u> mg/kg/day ^{a/}	<u>Route</u>	<u>Duration</u> weeks ^{b/}
Lee et al. (1975)	rat, mouse	--	oral	LD ₅₀
	rabbit	7.29%	dermal, ocular	72 hours
	guinea pig	3.41%	dermal	--
Lee et al. (1976)	dog	25, 50, 100, 200	oral	5 days
	dog	0.01, 0.1, 1.0 raised to 0.05, 0.5, 5.0	oral	4 9 ^{b/}
	rat (male)	0.8, 6.0, 59.0	oral	5
	(female)	0.9, 6.4, 59.3		
	(male)	2.6, 24.5, 229.5	oral	8 ^{b/}
	(female)	3.1, 26.5, 233.8		
	mouse (male)	1.3, 11.5, 106.7	oral	3
	(female)	1.3, 10.9, 94.9		
	(male)	6.4, 60.2, 607.6	oral	10 ^{b/}
	(female)	6.9, 58.7, 561.2		
rat (male)	1,406	oral	13	
(female)	1,416			
Ellis et al. (1978a)	dog	1, 5, 25	oral	52
	rat (male)	3.04, 31.5, 363	oral	104
	(female)	3.99, 38.1, 434		
mouse (male)	11.1, 115, 1020	oral	104	
(female)	9.7, 96, 1060			
Oketani et al. (1981a)	rabbit	0.5, 1.0, 4.0	intravenous	gestation days 6-18
Oketani et al. (1981b)	rat (male)	1, 10, 20	intraperitoneal	63 days prior to mating
	(female)			14 days prior to mating through gestation day
Oketani et al. (1981c)	rat	1, 10, 20	intraperitoneal	gestation days 7-17
Oketani et al. (1981d)	rat	1, 10, 20	intraperitoneal	gestation day through day 2 of lactation

^{a/} Unless otherwise stated^{b/} Continued for additional weeks

> Acute Toxicity:

Oral acute toxicity studies were conducted in mice and rats. The drug was mixed with lactose and suspended in peanut oil.

The acute oral dose in rats was reported at 822 ± 54 (700-953) mg/kg NTG in M and, 884 ± 61 (763-1055) mg/kg NTG in F. In mice the acute oral dose was reported, in M and F, respectively as 1188 ± 76 (1008-1352) and 1055 ± 63 (895-1178) mg/kg NTG.

In both species, the signs of toxicity appeared within 1-hr of treatment and included depressed respiration, cyanosis and ataxia, pallor was noted on the ear, nose, eyes, paws and tail. Animals that survived recovered within 24 hrs. No gross pathology was noted in the animals that died.

> Repeat Dose Studies:

◆ MOUSE

Briefly, in a 13-wk mouse study, the animals were treated orally with daily doses of NTG; the doses were increased after 4 wks of treatment because of the lack of adverse effects of NTG. At the end of the study, the MD/HD groups were allowed a 4-wk recovery period.

The achieved doses ranged from LD-0.005 up HD-0.5% NTG in diet; the estimated HD represents an average intake (M + F) of ≈ 558 mg/kg/day. The MD/HD F (after a 4-wk recovery period) showed an increase in absolute/relative kidney weights when compared to controls. At necropsy, the HD M showed focal interstitial nephritis and most F showed chronic murine pneumonia, subacute inflammation in the liver and/or chronic interstitial nephritis. Since absolute/relative spleen weight tended to increased vs controls; the lowest adverse effect level (LOAEL) was considered by drug sponsor to be the LD (≈ 0.005 % NTG in diet or ≈ 6 mg/kg/day).

Oral Carcinogenicity Study: In a 24-mo study dietary study, the mice were treated daily with oral doses of NTG of 0, LD-0.01%, MD-0.1% and HD-1% in diet; the average (M + F) drug intake was estimated, respectively, as ≈ 10.4 , ≈ 105.5 and ≈ 1040 mg/kg/day. Signs of toxicity reported at the HD included decreased food consumption/body weight gain, methemoglobinemia (resulting in the appearance of Heinz bodies), anemia, reticulocytosis, and hepatocellular dysplasia (noted a 12-mo interim sacrifice in all 4 HD M examined. The number of mice sacrificed were considered a low number by investigators. The noted lesions were considered a "normal degenerative aging change" in these mice. Various tumors (bronchoalveolar adenoma, cystadenoma and follicular cell tumor of the ovaries and chromophobe adenomas of the pituitary) were reported but were not considered by the authors "to be associated with" NTG intake. Reviewer noted no evidence of dose-related effect in the reported tumors. A high mortality was reported by 18 months into the study, and by 24 mo no HD mice had survived. In the

report, the authors stated that the "lack of sufficient animals for evaluation of testicular effects after 24 months feeding with [TNG] somewhat limits the usefulness of this study."

◆ RAT

In a 13-wk repeat-dose/interim sacrifice/reversibility study in rats, doses of NTG in diet were started at 0.001 up to 0.1%. Since no adverse effects were noted by 5 wks of treatment, the doses were then increased to 0.005 up to 0.5% for an additional 7 weeks. The only clinical chemistry reported was elevated SGOT levels in 2 HD M rats; the clinical chemistry change was reported as being reversible during the recovery period. At a 4-wk interim sacrifice, organ changes reported were increases in absolute liver/kidney weights in some MD/HD rats.

After 13 wks of treatment, although the findings followed no trend, decreases in the weight of some organs at MD/HD were noted (thyroid, heart and adrenals); HD rats showed increases in liver, kidneys and gonads. The no-observed adverse effect level (NOAEL) for NTG was not determined in this study because of the increase in relative kidney weight at the LD (estimated average intake of < 3 mg/kg/day NTG for 13 wks).

In a separate 13-wk rat study, 2.5 % NTG was mixed with 25% lactose), the average intake of NTG was estimated as 1416 mg/kg/day for F and 1406 mg/kg/day for M. NTG treated rats showed a decrease in feed intake and reduced body weight between wks 8 and 13 of the study. Histopathologic changes reported in these rats was mild to moderate atrophy of testis and, severe aspermatogenesis in M rats. Lactose treated rats showed increase in cecum weight.

Oral Carcinogenicity Study: In a 24-mo rat study (at 12-mo with recovery period of 4-wks and interim sacrifice), rats received NTG at 0.01, 0.1 and 1% in diet (average intake for M + F was estimated to be, respectively, ≈ 3.5 , ≈ 34.8 and ≈ 397.5 mg/kg/day NTG). At 24-mo, surviving rats were then allowed to recover for another 4 wks before final sacrifice.

Toxic signs reported included methemoglobinemia at HD; methemoglobinemia gave rise to anemia and increase in erythropoiesis with variable incidence of reticulocytes. Some HD rats showed increases in serum enzymes (increases in alkaline phosphatase, SGOT and SGPT). Unscheduled deaths were reported in all groups; some accidental and some M showing subcutaneous tumors or pituitary adenomas.

At the 12-mo interim sacrifice rats showed variable values in absolute/relative organ weights; HD rats tended to have higher organ weights vs controls. Recovery rats showed no methemoglobinemia; when sacrificed the organs of the drug-treated rats were within the range of the controls.

At the 24-mo sacrifice, organs weights were also variable; since the HD rats showed decreased body weight resulting in increased relative organs weights with the livers being the most prominently increased relative weights. HD rats showed pigmentation in spleen as well as in the epithelium of some rats. Histopathology of the liver from HD rats showed mild to severe cholangiofibrosis and hyperplasia of bile ducts. Cholangiofibrotic lesions became more severe in the rats that were allowed to recover for 4 wks and, showed a progressive development of hepatocellular carcinoma (also reported in some MD rats). Investigators considered that the hepatocellular carcinomas were drug related. Occasional metastatic lung nodules were noted in some rats; this was considered to be originating in the hepatocellular carcinomas. At HD, ~ 50% of the M rats were reported with interstitial cell tumors (also reported in some controls and LD rats). Tumors within the tunica albuginea produced pressure on the tubules causing atrophy and aspermatogenesis. Not considered drug related were hepatic hemangiosarcomas noted in 3 HD M.

The following 3 tables were provided in this NDA showing the incidence of mortality, significant pathology in rats dying during the 24-mo NTG feeding study and, that in rats that survived and were necropsied after the 24-mo of treatment.

Incidence of Mortality in Rats Fed TNG for 24 Months^{a/}

Weeks (Dose mg/kg/day)	Male				Female			
	Control	0.01X	0.1X	1X	Control	0.01X	0.1X	1X
0	304	31.5	363		0	3.99	38.1	434
52	1 ^{b/}	4	1	0	1	2	1	1
53-78	5	2	3	2	2	2	4	1
79-91	2	8	2	3	6	9	8	2
92-104	9	7	7	5	8	8	4	0
105-108	0	3	0	0	0	0	1	0
Total Deaths	17	24	19	10	17	21	18	4
Total Sacrificed	15	10	20	19	20	19	18	29
Number ^{c/} Unaccounted For	6	4	5	7	1	24 ^{d/}	2	5

^{a/}Reference: Ellis et al., 1970a.
^{b/}Number of animals/clin period; includes only those animals dying and necropsied during the course of the experiment.
^{c/}Assumed to be found dead and discarded.
^{d/}Starting no. = 28/sex/level. Assume subsequent loss for early deaths included.

Rat Histopathologic Findings (Cont'd)

Significant Pathology in Rats Dying During TNG Feeding Study^{a/}

Weeks (Dose mg/kg/day)	Male				Female			
	Control	0.01X	0.1X	1X	Control	0.01X	0.1X	1X
	0	3.04	31.5	363	0	3.99	38.1	434
No. Dying ^{b/} during study	17	24	13	10	17	21	18	4
X Hepato- cellular Carcinoma	0	0	15(2) ^{c/}	50(5)	0	0	0	0
X Neo- plastic Nodules	6(1)	0	0	10(1)	0	0	0	75(3)
X Hyper- plastic Foci	24(4)	50(12)	62(8)	60(6)	24(4)	24(5)	61(11)	50(2)
X Testi- cular Tumors	0	0	8(1)	30(3)	-	-	-	-

a/ Reference: Ellis et al., 1978a.

b/ 38/sex/level at start of experiment.

c/ (n) = actual number of animals with reported pathology.

Survival Data and Significant Pathology in Rats
Necropsied After 24 Months of TNG Feeding

Weeks (Dose mg/kg/day)	Male				Female			
	Control	0.01X	0.1X	1X	Control	0.01X	0.1X	1X
	0	3.04	31.5	363	0	3.99	38.1	434
No. b/ Necropsied at 24 Months	4(3) ^{c/}	4(3) ^{d/}	9(4)	8(3)	9(3)	7(4)	7(4) ^{e/}	10(3)
X Hepato- cellular Carcinoma	0	0	11(1) ^{f/}	75(6)	0	0	14(1)	56(10)
X Neoplastic Nodules	0	0	0	12(1)	0	0	14(1)	11(2)
X Hyper- plastic Foci	25(1)	50(2)	67(6)	38(3)	22(2)	20(2)	86(6)	83(15)
X Testi- cular Tumors	25(1)	25(1)	22(2)	75(6)	-	-	-	-

a/ Reference: Ellis et al., 1978a.

b/ 38/sex/level at start of experiment.

c/ (n) = number of animals necropsied for pathology study.

d/ All animals died before completion of recovery study.

e/ An animal died before completion of recovery study.

f/ (n) = actual number of animals with reported pathology.

From the findings in this rat study, it may be concluded that repeated doses of NTG in rats was associated with methemoglobinemia, anemia, compensatory erythropoiesis with variable incidence of reticulocytosis, large livers, hepatocellular carcinoma and testicular tumors (~ 52% of the HD M showed interstitial cell tumors in testes vs up to ~ 12% incidence in LD and ~ 8% in controls). At the highest doses tested, liver histopathology showed cholangiofibrotic lesions; a progressive development of cholangiofibrotic lesions and hepatocellular carcinomas were noted during the 1-mo recovery period. Occasional metastatic lung nodules were noted in some rats, but these were considered to be originating in the hepatocellular carcinoma.

◆ DOG

In a 5-day dog, the highest dose tested of 200 mg/kg/day (capsules) was associated with methemoglobinemia usually within 1 hr of treatment; the methemoglobinemia lasted up to 24 hr (last measurement); injection of methylene blue offered some protection against the rapid formation of methemoglobinemia. Beagles treated with NTG in capsules containing 0, 0.01, 0.1 or 1 mg/kg/day for 4 wks, showed no adverse effects. Since these doses of NTG did not induce any observable adverse effects, the dose was increased to 0.05, 0.5 and 5.0 mg/kg/day for 13 wks; again no observed adverse effects were reported with the treatment of NTG.

In another dog study, animals were treated with 1, 5 and 25 mg/kg/day (in capsule) designed to last for 2 years, the study was terminated at 1 year (+ 1 mo for observation) because of methemoglobinemia. Histopathology findings were reported as showing no remarkable toxicologic findings. (No testicular tumors were reported.) (Ellis et al, -1978)

◆> REPRODUCTIVE TOXICOLOGY:

◆ RAT

In a rat 3-generation oral reproduction study, the F_0 rats were fed NTG (0, 0.01%, 0.1% and 1.0% in the diet; actual drug intake reported for M as ~ 3.4, 31.5 and 363 mg/kg/day and for F as ~ 3.99, 38.1 and 434 mg/kg/day) up to 6 month prior to mating, throughout mating, during resulting pregnancy and through weaning of their F_{1b} offspring (2nd litter). The selected F_{1b} offspring were then fed NTG in diet during growth and subsequent production of a F_{2b} generation and, selected litters through weaning of their F_{2a} and F_{2b} litters.

The results reported indicate that at the highest dose tested, NTG treatment caused decreased mean body weights but no specific effect on fertility on the F_0 generation. In the subsequent generations (F_1 and F_2), fertility was reduced in the HD groups; reduced litter size and incomplete ossification were reported in these F_1/F_2

generations. Microscopic examination of the HD F₂ M revealed aspermato-genesis considered due to increased interstitial tissue and smaller testicular size. When the HD F₂ F were mated with untreated M, this resulted in higher pregnancy ratio (13 of 14 F became pregnant) suggesting that the previously noted reduced fertility in the HD F₂ generation was due to the drug treated M.

The teratogenic effects of NTG in rats was said to have been studied in F₀ F rats from the 3-generation study that had been mated for the 3rd time. The HA summary states that developmental anomalies in soft tissues (1 diaphragmatic hernia) and skeleton [statistically significant (P<0.05) increase in the incidence of absent/incomplete ossification of the hyoid bone vs control] were reported in the HD group at ~ 434 mg/kg/day NTG. The skeletal anomalies were considered to be indicators of delayed development and were not normally indicative of teratogenic potential.

◆> IMMUNOTOXICOLOGY:

Limited studies were conducted in dogs and rats. HA reports that rats (2.5% NTG in diet) and dogs (0.1 up to 5.0 mg/kg/day NTG in diet) each species for 13-wks showed no increase in serum conc of IgE antibodies; dogs showed no sensitization or allergic reactions when compared to controls (Lee et al 1976).

◆> PHARMACOKINETICS/TOXICOKINETICS:

The literature reports that in humans, plasma levels of NTG have been measured following various routes of the drug administration. However, the authors state that drug evaluations based on plasma levels must be viewed with caution because of the rapid disappearance of the drug from plasma. Further, that after sublingual administration of NTG, plasma levels of the drug tend to be higher than after oral administration suggesting a high first-pass extraction after this route.

In experimental animals, the drug is reported as being well absorbed orally and undergoing first pass-metabolism primarily in the liver which is reflected in a ~ 38% ± 26% bioavailability in humans but there is considerable inter subject variability is reported after sublingual administration of the drug.

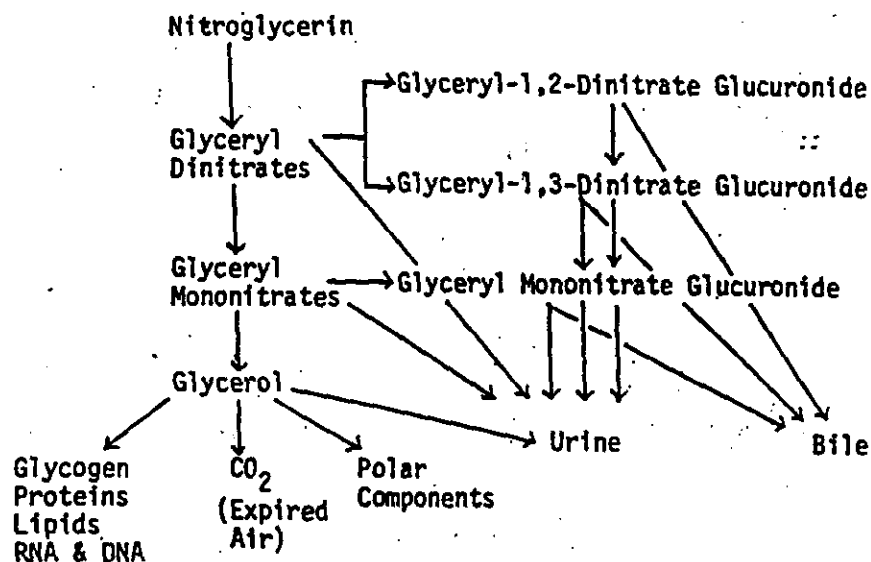
The pharmacokinetics/metabolism was extensively summarized in the HA. However, the present brief overview was prepared from information extracted mainly from the Labeling Update for Nitrodisc because the data described therein were based on the original study reports prepared for the US Army.

Briefly, in vivo metabolism studies with NTG were conducted in mice (A albino Swiss), rats (CD), rabbits (New Zealand W), beagles and monkeys (rhesus). Animals were fasted overnight prior to dosing with a single oral dose of ^{14}C -NTG. After dosing, expired air, feces and urine were collected. At the end of the study, animals were anesthetized and their blood collected and at sacrifice, tissues were removed, weighed and digested. Extracts were prepared from samples collected, prepared/counted. Various techniques were used to identify NTG and its metabolites.

Within 24 hrs of administration ^{14}C -NTG, ~ 91-98 % of the administered radioactivity was recovered in tissue and excreta of rabbits and dogs and, ~78-83% in the other species studied (mice, rats and monkeys) suggesting that the drug was well absorbed. Regarding excretion in feces, ~ 28% of the administered dose was excreted by mice and 0.1% up to 6.3% was excreted by the other species studied. At the end of 24 hrs, the liver of all species studied contained levels of radioactivity of within the same range (~ 4% in rat up to ~ 7 % in monkey).

Less than 1% of unchanged NTG was accounted for in urine. Reports indicated that the metabolic pathway of orally administered NTG was essentially the same in all species studied.

Although several metabolites of NTG have been characterized the their distribution studied, no schematic representation of the proposed metabolic pathway of NTG could be located in the NDA. The following pathway was published extracted from the literature*.



Metabolic Fate of Nitroglycerin

* EF McNiff et al in "Nitroglycerin", Analytical Profiles of Drug Substances, V 9, edited by Klaus Florey, Academic Press, 1980

In fasted anesthetized F rats, biliary excretion of NTG and its metabolites was studied using animals with common duct cannulation. The rats were given the tests compounds by gavage. Bile and blood samples were collected at variable intervals up to 24 hrs. After 24 hr, the animals were sacrificed and blood and gi tract/fecal samples were collected for measuring the radioactivity levels.

In the study, radioactivity appeared in the bile within 15 min after dosing; reached a peak in 3 hrs and decreased thereafter. By 24 hrs, total biliary excretion of administered radioactivity averaged 13% of the dose; blood levels of radioactivity continued to increase in the rats for 5-6 hrs postdosing and then decreased.

In the bile, small levels of the unchanged drug were detected. The levels of the metabolites in bile were in the following order: 1,2-DNG glucuronide > glycerol > polar compounds > 1,3-DNG glucuronide > MNG > 1,3-DNG > 1,3-DNG.

The in vitro metabolism of NTG was investigated using liver homogenates obtained from various species (human, mice, rabbits, dogs and monkeys). Reports indicate that the drug is rapidly metabolized to DNGs metabolites. No sex difference in metabolism in these species was reported. Human, dog, rabbit and monkey livers produced more 1,2-DNG than 1,3-DNG while rat livers produced more 1,3-DNG than 1,2-DNG. Compared to liver, some organ/tissues homogenated (i.e., placenta from mice, rats or human and mouse embryo, liver or carcass during late gestation) had a poor ability to metabolize NTG in vitro.

NTG metabolism by rat liver appeared to change with age since the ability of rat liver to metabolize ability at day 21 was lower than that of the adult rat.

No protein binding studies were reported. However, the presently approved label for NTG drug products report the results of protein binding studies.*

Strain	Histidine mutation	uvrB excision repair	pKM101
TA1538	hisG46	-	-
TA100	hisG46	-	+
TA102	hisG421	+	+
TA1535	hisD3052	-	-
TA1975	hisG46	+	-
YG1026	hisG46	-	+
TA100NR	hisG46	-	+

* "At plasma concentrations of between 50 and 500 ng/mL, the binding of nitroglycerin to plasma is approximately 60%, while that of 1,2 dinitroglycerin and 1,3 dinitroglycerin is 60% and 30%, respectively."

◆> GENETIC TOXICOLOGY:

In vivo (dominant lethal test) and vitro (bacterial/mammalian gene mutation) assays were conducted to determine the genotoxic potential of NTG. All assays incorporated positive controls which were reported as showing the appropriate response.

A reliable indicator of dominant lethality is a statistically significant increase in the number of early embryonic deaths (dead implantations) in F mated with drug treated M.

>In the dominant lethal test, M rats (CD came from a 13-wk repeat dose study with NTG at 0, 0.01, 0.1 and 1% in feed) were mated with untreated F. At mid-term of pregnancy the F were killed and their reproductive parameters examined to determine damage (e.g., mating performance, number pregnant, corpora lutea, total implantations and viable implants).

The results reported indicate that there was no evidence of adverse effect on the M fertility or on any of the reproductive parameters of F examined. The authors concluded that NTG showed no dominant lethal mutation in this assay.

> In vitro the bacterial cell gene mutation assays, 5 different *S. typhimurium* tester strains were examined: TA98, TA100, TA1535, TA1537 and TA1538.

The results reported indicate that NTG displayed a weak mutagenic activity in TA1535 and TA1537 at the highest conc tested (1000 µg/plate. Under conditions of the test 1000 µg/NTG/plate was needed to produce a notable increase in mutation frequency in TA1535 (with metabolic activation with rat liver S-9 fraction) and in TA1537 (without metabolic activation). No remarkable effects were noted in these two strains at the 3 lower conc tested of NTG (10, 100 and 300 µg/ml) or at any conc studied in the other strains used in the assay (TA98, TA100 and TA1538).

> In a published bacterial cell mutagenicity study appearing in a journal article submitted in the NDA (V.1; Ref. 7), CM Maragos et al reported** the effects of NTG using the *S. typhimurium* strains listed below.

* TA100 and TA1535 detects base-pair substitutions and, TA98, TA1537 and TA1538 to detect frame-shift reverse mutation.

** CM Maragos, et al in "Mutagenicity of glyceryl trinitrate (nitroglycerin) in *Salmonella typhimurium*", Mutation Research 298 (1993) 187-195.

The NTG used was obtained from sublingual tablets (formulated with lactose) and a solution used for iv administration (dissolved in alcohol/propylene glycol/water) that were provided from two different firms. When tested, NTG/lactose was dissolved in DMSO.

The journal article reported the materials/methods used and listed the positive/negative controls tested (methyl methanesulfonate, 2-nitronaphthalene, 2-aminoanthracene and sodium azide and DMSO as negative control). For metabolic activation, the S-9* fraction used was obtained livers from Aroclor treated hamsters.

Briefly stated, the results reported indicate that NTG (from both sources) showed weak mutagenic activity in the S. typhimurium strain TA1535 (the repair-deficient uvrB and lacking in PKM101 which is responsive to single base changes) as evidenced by the increased number of His+ revertants. The drug exhibited cytotoxicity in the other strains tested. The excipient lactose (used in the sublingual tablets) did not exhibit mutagenic effect.

The authors referred to the work of another investigator which reported nitric oxide gas (NO) to be mutagenic in TA100 and to inhibit DNA synthesis in some cell types as does GTN*.

The present authors conducted intricate assays (using oxymyoglobin as a scavenger for NO), and reported that they were able to demonstrate that the mutagenicity of GTN does not result from exposure of extracellular NO, and concluded that there may be considerable variability in the vulnerability to GTN-induced mutagenesis. They speculated on the influence of NO derived from NTG by metabolic reduction on the observed mutagenic effect, but concluded by putting forward the hypothesis that intracellular NO is responsible for the observed mutation. They stated that strains proficient in either uvrB excision repair (TA1975) or PKM101-mediated repair (TA100, TA100NR, YG1026) or both (TA102) failed to show mutagenesis by NTG, but were at least as susceptible as strain TA1535 to its toxic effects, and suggested that the mutagenic damage is efficiently repaired by some strains, while the cytotoxicity is not reduced or is enhanced by DNA repair. TA102 (exhibiting both repair systems) exhibited the sharpest decline in colony number.

> In an in vitro assay to detect gene mutation in mammalian cells, NTG was tested using wild type CHO (CHO-K1); the wild type cells are capable of growth in minimal and enriched medium. These cells

* KM Isomura in "Induction of mutations and chromosome aberrations in lung cells following in vivo exposure of rats to nitroxy oxides", Mutation Res 136:119-25, 1984.

were exposed to two conc of NTG (50 and 144.8 µg/ml; selected from a single cell survival curve), according to the method of Puck and Kao (1965).*

The report states that mutant CHO-K1 cells are capable of growth only in enriched medium and not in minimal medium. This assay does not incorporate metabolic activation. In the assay, mutagenesis was measured relative to the positive control and mutagen ethyl methanesulfonate.

The results reported indicated that the conc of NTG tested killed, respectively, 65% and 99% of the cell population but showed no evidence of gene mutations; the positive control clearly induced mutant cells. Regarding the results, the investigators asserted that since this assay does not incorporate a metabolic activating system, the assay yields no information on the mutagenicity of the NTG metabolites.

> LABELING:

The proposed Labeling appears adequate. No changes are recommended at this time because the labeling language is that previously recommended by this Division for other NTG drug products.

> EVALUATION:

NTG is a marketed drug indicated for the prophylaxis or acute relief of angina pectoris due to coronary artery disease and is marketed in the form of several drug formulations including sublingual tablets. The drug has been used for over 100 years. Some sources credit W Murrell with establishing the use of sublingual NTG for relief of acute angina pectoris late in XIX century. Other sources give credit for the use of NTG, as a classical coronary vasodilator to W Evans and C Hoyle (1934) and HK Russek (1950). The present NDA is for a reformulated compressed and already marketed sublingual tablets of NTG.

No nonclinical studies were conducted for this NDA. Prior to this submission, safety studies with NTG had been conducted by mixing the drug in the diet of animals to investigate the short-term, long-term (including carcinogenicity), reproduction, pharmacokinetics of the drug. Also, in vitro/in vivo studies had been conducted to investigate the genotoxic potential of NTG. None of these nonclinical studies were conducted under GLP regulations. The studies were conducted between 1973-1985 under contract for the US Army and were evaluated in DCRDP in 1993 to determine whether the data supported a proposed labeling update for a marketed transdermal system.

* A reference for this assay was not provided. The following reference was identified in the literature: Kao, FT and Puck, TT in "Genetics of Somatic Cells: Mutagenesis by Carcinogenic Compounds" in J. Cell Physiol., 78:139-44, 1971.

Toxicology: In a 13-wk study in mice, doses up to 0.5% in diet were well tolerated; the HD dose (-560 mg/kg/day) was associated with subacute inflammation in the liver, interstitial nephritis and extramedullary hematopoiesis. In a 24-mo mouse study, the HD (-1040 mg/kg/day) was associated with methemoglobinemia, anemia, reticulocytosis, and hepatocellular dysplasia (noted at a 12-mo interim sacrifice. Except for the hepatocellular dysplasia, pathologies were reversible. The liver lesions were considered by the investigators to be due to normal degenerative age changes.

In a 13-wk study in rats, doses up to 0.5% NTG in diet were well tolerated. In a separate study, doses up to 2.5% NTG for 13 wks revealed drug related atrophy of testis and depressed spermatogenesis and hemosiderosis in the spleen and bile duct proliferation in the dams. In a 24-mo rat study, the HD (at 1% NTG in diet and average intake of - 400 mg/kg/day), rats showed methemoglobinemia, anemia, compensatory erythropoiesis with variable incidence of reticulocytosis, large livers, hepatocellular carcinomas and testicular tumors. The liver histopathology of HD rats included mild to severe cholangiofibrotic lesions (hyperplasia of bile ducts); there was progressive development of this lesion and hepatocellular carcinomas even in the rats that were allowed to recover from drug-treatment for 1-mo. Occasional metastatic lung nodules were also noted in some rats, but these were considered originating in the hepatocellular carcinomas.

In dog, doses of 1-25 mg/kg NTG/day for 1 year was associated with methemoglobinemia. No histopathologic changes related to the drug were reported.

Reproduction Studies: In three-generation oral reproduction study, no adverse effects were reported on fertility in the F₀ rats that had been treated with NTG (from 0.1 up to 1.0% in diet for 6-mos prior to mating). Reduced fertility was reported at the HD (M/F = 363/434 mg/kg/day) rats in the F₁ and F₂ generations. However, when the HD F₂ dams were mated with untreated control M rats, 13 out of 14 F became pregnant; this finding suggested to the investigators that reduced fertility previously noted was due to the drug treated M (whose testes revealed aspermatogenesis and mild to moderate increased interstitial tissues). In the teratology portion of this 3-generation study, examination of the fetuses from of HD F₀ dams that had been previously mated with untreated M (and according to the protocol treated on days 6-15 of pregnancy) showed diaphragmatic hernia (4 of 19 litters). Some of the dams showed increased liver weight.

Genotoxicity Studies: Studies were conducted prior to the publication of our present guidance. Thus, some of the assays might be considered unreliable (e.g., analysis of bone marrow/kidney cells long term studies with NTG in rats/dogs) and are not evaluated here.

The in vivo dominant lethal test is not recommended, in our present standard battery for genotoxicity testing of pharmaceuticals, but it is a test to detect chromosome aberrations and is applicable to germ cell risk. However, some investigators have also reported that the test is relatively insensitive.* The NDA reported that for the assay M rats used were those that had been treated with NTG (0.01 up to 0.13% in diet/day) for 13-wks. These drug-treated M were mated with untreated virgin F and their maternal parameters examined.

No adverse effect was reported on M fertility or in the dams on the number of implantations/dams or implant viability. The authors concluded that results suggest that NTG did not show a dominant lethal mutagenic effect in this in vivo assay.

In the test to detect gene mutation in bacterial cells, NTG (at the highest conc tested; 1000 $\mu\text{g}/\text{plate}$) was reported as showing weak mutagenic activity in 2 (TA1535 and TA1537) out of the 5 tester *S. typhimurium* strains assayed. An increase in "mutagenic ratio" (# of histidine revertants in the test culture/number of histidine revertants in control dish) was reported for NTG in the presence of S9 metabolic activation for TA1535 and, in the absence of S9 activation in TA1537 strain.

The drug sponsor provided a journal article (V.1; Ref. 7) in which the authors revisited the potential for mutagenicity effect by NTG (obtained from 2 pharmaceutical preparations). The drug was tested in the *S. typhimurium* strain TA1535 and other strains (see genotype/sources above under GENOTOXICITY). In this study, the metabolic activation S9 liver fraction used was obtained from hamsters treated with Aroclor 1254. The investigators also used a so-called nitric oxide (NO) scavenger to determine the source of the NO generated during the assay (e.g. "extracellular" or hypothesized from "intracellular" sources).

Although TA1535 was the only strain of those tested showing a mutagenic response to NTG (from each of the two sources: 2.5 up to 7.5 $\mu\text{mol}/\text{plate}$ without metabolic activation or at 2.2 $\mu\text{mol}/\text{plate}$ with/without metabolic activation), the authors interpreted the results of the studies to show that the mutagenicity of NTG "does not result from exposure to extracellular nitric oxide" and added that the data "are consistent with the hypothesis that intracellular nitric oxide is responsible for the observed mutations." The significance of these findings are difficult to interpret.

* SS Epstein et al "Detection of chemical mutagens by the dominant lethal assay in mouse", Toxic Appl Pharmacol 23:288-325, 1972 cited in "The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity" September 1973, Health and Welfare of Canada, Sante et Bien-etre Social Canada.

In the assay to detect gene mutation in mammalian cells using CHO-K1 cells, NTG (at a selected conc of 50 or 144.8 µg/ml) showed no genotoxicity. Since this assay does not include metabolic activation, the authors pointed out that the assay yielded no data on the potential genotoxic activity of the metabolites of NTG.

In conclusion, numerous coronary dilator drugs have been introduced and discarded, but NTG has been continuously used in the prophylaxis and relief of angina pectoris. The nonclinical data available on NTG show weak evidence of gene toxicity, at the highest conc tested, in 2 strains of *S. tiphymurium* (TA1535 and TA1537); equivocal effects on fertility in rat, and clear evidence of testicular tumors (accompanied by atrophy and aspermatogenesis) and hepatocellular carcinomas in rats.

Based on the long clinical use and wide experience with this marketed drug and, in the absence of epidemiologic data on the tumorigenic risk to humans, it may be unreasonable to expect a commercial firm to repeat nonclinical studies that have shown equivocal findings (e.g., bacterial genotoxicity). The findings in the mutagenicity and carcinogenicity assays are adequately disclosed in the label. Furthermore, evidence to date suggest that the findings in these assays are mediated through NO.

RECOMMENDATIONS: PHARMACOLOGY considers this NDA approvable including the drug sponsor proposed labeling language in the sections on "Carcinogenesis, Mutagenesis, Impairment of Fertility" and "Pregnancy Category C."

cc
Orig NDA
HFD-110
HFD-110/RHPM
HFD-110/EAGBarry
HFD-345

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ADP: 7/20/99