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Provisional Peer Reviewed Toxicity Values for

Nitroglycerin
(CASRN 55-63-0)

Derivation of Subchronic and Chronic Oral RfDs

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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion

ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR NITROGLYCERIN (CASRN 55-63-0)
Derivation of Subchronic and Chronic Oral RfDs

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

A reference dose (RfD) for nitroglycerin (also known as trinitroglycerol) is not available on the Integrated Risk Information System (IRIS) (U.S. EPA, 2006) or in the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) does not include an RfD for nitroglycerin, but does include 1-day, 10-day, and lifetime drinking water health advisories of 0.005 mg/L based on a mean therapeutic dose of 0.005 mg/kg-day used for treatment of acute angina (U.S. EPA, 1987). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994) does not include any other review documents for nitroglycerin. Neither the Agency for Toxic Substances Disease and Registry (ATSDR) (2005), National Toxicology Program (NTP) (2005), International Agency for Research on Cancer (IARC) (2005) nor the World Health Organization (WHO) (2005) has produced any documents regarding nitroglycerin. Literature searches were conducted from 1965 to July 2003 in TOXLINE (including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS. Update literature searches were performed in August 2005 in MEDLINE, TOXLINE (NTIS subfile), TOXCENTER, TSCATS, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS and Current Contents.

REVIEW OF PERTINENT LITERATURE

Human Studies

Contact with nitroglycerin can occur through occupational exposure in the explosives industry or through its medical use as a vasodilator in the relief of angina pectoris. Adverse effects caused by vasodilation include hypotension, reflex tachycardia, cardiac arrhythmias, headache, dizziness and nausea. Key issues that must be considered in deriving toxicity values for nitroglycerin are (1) acute vasodilation in persons exposed to nitroglycerin therapeutically as discussed below or in combination with other compounds in the workplace, (2) the rapid development of tolerance to the vasodilatory effects of nitroglycerin with repeated exposures (therapeutic dosage regimens are designed to avoid continuous exposure to nitroglycerin and include daily nitrate-free periods), (3) the lack of accumulation of nitroglycerin in the body due to its short half-life, (4) the association of chronic exposure to the compound with organic nitrate dependence, in which withdrawal from the treatment can cause severe rebound hypertension leading to (occasionally fatal) heart attack and stroke in subjects who may have adapted to the continuous presence of the compound to maintain optimum cardiovascular function and (5) the potential for the compound to be associated with systemic toxicity as indicated by long-term exposure studies in laboratory animals.

Therapeutic Use of Nitroglycerin

Aliphatic nitrates, such as nitroglycerin, have been used therapeutically for over 100 years in the treatment of myocardial ischemia (angina pectoris), providing an extensive clinical experience (Kerins et al., 2001). Aliphatic nitrates are used to control angina pectoris because exposure to them causes vasodilation, thereby, lowering blood pressure and reducing the cardiac workload. The mechanism of vascular smooth muscle relaxation is brought about by the generation of nitric oxide, a potent vasodilator (Katzung and Chatterjee, 2001; Kerins et al., 2001; NIOSH, 1978). In developing treatment regimens, nitroglycerin and other aliphatic nitrates are prescribed for intermittent use with dose levels established by the degree of relief from symptoms afforded to the patient as the nitroglycerin amount is raised. This approach reflects the rapid onset of the compound's pharmacological effects, rapid development of tolerance and the marked individuality of response to treatment among different patients (Abrams, 1980a).

Aliphatic nitrate compounds are administrated by the oral, sublingual, buccal, dermal and intravenous routes. Sublingual administration is the preferred route for treatment of acute anginal attacks, due to the rapid onset of action and avoidance of extraction of orally administered nitroglycerin through hepatic metabolism. In clinical medicine, oral nitroglycerin is used for long-term control of angina pectoris and is only administered as sustained- or extended-release preparations designed to provide stable blood levels and therapeutic efficacy for 6-8 hours (Katzung and Chatterjee, 2001). Based on data available in the clinical literature, there is no information to determine the minimum effective dose of nitroglycerin. The lowest available (i.e., marketed) single oral dose of sustained-release nitroglycerin is 2.5 mg (equivalent to 0.036 mg/kg-day, assuming a typical body weight of 70 kg) with a recommended maximum total daily oral dose of 38 mg/day (Katzung and Chatterjee, 2001; Kerins et al., 2001). Doses administered by non-oral routes are generally lower than those used for oral administration since non-oral routes avoid first pass metabolism. Due to the high first-pass hepatic extraction of

orally administered nitroglycerin, extrapolation of dose-response relationships observed for intravenous, sublingual, buccal or dermal routes cannot be readily extrapolated to the oral route. Thus, only information pertaining to the oral route of administration is considered quantitatively applicable to the development of an oral RfD.

Estimates of the oral bioavailability of nitroglycerin range from <1% (Kerins et al., 2001) to <10-20% (Katzung and Chatterjee, 2001). Active metabolites of nitroglycerin (1,2- and 1,3-dinitroglycerol) are responsible for some, if not all, of the vasodilatory effects following oral administration since first-pass metabolism eliminates nearly all absorbed nitroglycerin before it can be detected in the systemic blood (Kerins et al., 2001; Kwon et al., 1992). Compared to nitroglycerin, the nitroglycerol metabolites appear to be less potent and longer-lived in the plasma (Katzung and Chatterjee, 2001; Kerins et al., 2001). Plasma half-life estimates for the dinitroglycerols range from 40 minutes (Kerins et al., 2001) to up to 3 hours (Katzung and Chatterjee, 2001), compared to 2-9 minutes for nitroglycerin (Katzung and Chatterjee, 2001; Kerins et al., 2001).

The extent of clearance of nitroglycerin administered orally was examined by Laufen and Leitold (1988), who administered 20 mg of oral "sustained-release" nitroglycerin (Nitro Mack: Retard) to six human volunteers. Nitroglycerin and its metabolites were measured in serial blood samples taken between 10 minutes and 28 hours after dosing. No unchanged nitroglycerin was observed in plasma, indicating the importance of first-pass metabolism in the clearance of this compound from the circulation. Plasma concentrations of the mono- and dinitrate metabolites peaked at about 5 hours. Kwon et al. (1992) used a similar experimental protocol to compare the efficacy of sustained-release nitroglycerin versus oral solution or sublingual doses of nitroglycerin. There were eight healthy volunteers who participated in a crossover study in which the various dosings were separated by a 3-day "washout" phase. Serial blood samples were collected at 10 minute intervals for the first 40 minutes then hourly to 12 hours and at 14, 16, 20 and 24 hours after dosing. Periodic measurements of blood pressure were also made within the same time frame. Unchanged nitroglycerin was detected in blood after sublingual dosing, but not after the oral dosing, confirming the rapid and near-total clearance of nitroglycerin from the circulation after oral dosing. Kwon et al. (1992) found higher levels of 1,2-glyceryl dinitrate in the blood compared with the 1,3-isomer. The absence of unchanged nitroglycerin in the systemic circulation after oral dosing with nitroglycerin reported by Laufen and Leitold (1988) and Kwon et al. (1992) has been interpreted as evidence that the metabolites of nitroglycerin contribute to, and possibly account for, all the pharmacological activity of orally-administered nitroglycerin.

At therapeutic doses, regardless of the route of administration, adverse effects of nitroglycerin result from non-specific vasodilation, and include hypotension, reflex tachycardia, cardiac arrhythmias, headache, dizziness and nausea. Headache is commonly reported by patients treated with aliphatic nitrates and can be severe (Kerins et al. 2001). Effects can range in intensity from mild to severe, depending upon dose and individual susceptibility (Kerins et al., 2001). When nitroglycerin is administrated repeatedly, tolerance develops to the vasodilatory effect, leading to reduced therapeutic efficacy and a lessening of adverse effects (Abrams, 1980a). Due to the rapid development of tolerance, therapeutic dosage regimens are designed to avoid continuous daily exposure by providing a daily nitrate-free period of 8-12 hours (Kerins et al., 2001; Abrams, 2002). With an elimination half-time estimated to be approximately 2-9 minutes (Kerins et al., 2001), the nitrate-free interval is sufficiently long for essentially all

nitroglycerin to be eliminated from the body. Since tolerance reverses rapidly following a nitrate-free period, adverse effects associated with vasodilation redevelop upon subsequent exposures. The phenomenon of repeated redevelopment of acute adverse effects following short nitrate-free periods has been described for therapeutic (Katzung and Chatterjee, 2001; Kerins et al., 2001) and occupational (Daum, 1983) exposures to nitroglycerin. Attempts to overcome tolerance by dose escalation have consistently failed, with therapeutic efficacy restored only after nitrates are absent from the body for several hours (Kerins et al., 2001).

Nitroglycerin also has the capacity to cause dependence, whereby cessation of repeated exposure can cause rebound vasoconstriction that may be severe, leading to the development of hypertension, angina, weakness, palpitations, coronary artery vasospasm, difficulty in breathing and, in some cases, death (Abrams, 1980b; Daum, 1983; Kerins et al., 2001). For therapeutic use of nitroglycerin, rebound vasoconstriction is most commonly associated with continuous intravenous infusion over several days (Kerins et al., 2001).

The clinical experience with nitroglycerin is that methemoglobinemia can occur, but only at doses higher than those used in the treatment of cardiovascular diseases (Katzung and Chatterjee, 2001). Therapeutic doses of nitroglycerin were not associated with the development of methemoglobinemia following sublingual administration to healthy volunteers (Paris et al., 1986) or intravenous infusion to patients (Kaplan et al., 1985). Intravenous infusion of nitroglycerin to patients at rates approximately 10-fold higher than the recommended infusion rate of 5 µg/hr (Kerins et al., 2001) resulted in development of slight methemoglobinemia (Gibson et al., 1982; Husum et al., 1982). Although studies specifically investigating the ability of oral nitroglycerin to induce methemoglobinemia were not identified from the published literature, clinical studies using the sublingual and intravenous routes suggest that methemoglobinemia is only observed at nitroglycerin doses greater than those used therapeutically.

Occupational Exposure

Additional information on human exposure to nitroglycerin via the inhalation route is available from studies on occupationally exposed workers in the explosives industry (Abrams, 1980a; Craig et al., 1985; Daum, 1983; Kristensen, 1989). The findings of these studies demonstrate the importance of the development of rebound vasoconstriction in workers whose normally elevated organic nitrate body burden becomes depleted during a brief period of absence (1-2 days) from the workplace. The well-documented phenomenon of “Monday Morning Death” has been described for munitions workers in whom cardiovascular events can be triggered by unrestrained compensatory vasoconstriction as the organic nitrate in the body becomes reduced (Abrams, 1980a). Incidence of sudden death in such persons is up to 15 times the expected rate (Daum, 1983). Kristensen (1989) discussed an apparent double effect of nitroglycerin in cardiovascular disease, where the incidence of “Monday Morning Death” was compounded by an apparently increased risk of heart attack and stroke that continued in chronically exposed subjects long after the cessation of exposure.

Craig et al. (1985) carried out a death certificate study of persons who had been employed at an explosives factory in Scotland. The investigation covered the time period 1965 to 1980 and compared the causes of death of potentially exposed workers with internal controls (unexposed workers at the same factory) and members of the general male population. Blasting

workers (exposed to nitroglycerin and ethylene glycol dinitrate) and propellant workers (exposed to nitroglycerin alone) were stratified by age and then by high- and low-exposure subcategories, depending on the position of their work station or the nature of their duties. Causes of death for each target group included acute myocardial infarction (MI), ischemic heart disease (IHD), cerebrovascular disease, lung cancer, cancer of the colon or rectum and all cancers. Among the non-cancer related responses, there was an increased risk of MI and IHD in the 15-49 age-group of high-exposure blasting workers compared with internal controls or with members of the general population, with six deaths observed in the high-exposure blasting group compared to three expected deaths. However, these findings do not necessarily implicate nitroglycerin as no effect was reported among propellant workers exposed to nitroglycerin alone.

Animal Studies

Ellis et al. (1984) conducted a chronic toxicity study with nitroglycerin in dogs, rats and mice. In this study, beagle dogs (6/sex/group) were administered daily capsules that provided doses of 0, 1, 5 or 25 mg nitroglycerin/kg-day for 12 months. Groups of CD rats (38/sex/group) and CD-1 mice (58/sex/group) were fed diets containing 0, 0.01, 0.1 or 1% nitroglycerin for up to 24 months. The intakes of nitroglycerin estimated by the investigators for male and female rats, respectively, in the treated groups were 3.04 and 3.99 mg/kg-day, 31.5 and 38.1 mg/kg-day and 363 and 434 mg/kg-day. For mice, the nitroglycerin intakes for male and females, respectively, in the treated groups were 11.1 and 9.72 mg/kg-day, 114.6 and 96.4 mg/kg-day and 1022 and 1058 mg/kg-day. During the first several weeks of exposure all animals were observed daily for clinical signs of toxicity, with none observed. Animals were not assessed for vasodilatory effects. Since all animals were sacrificed at the end of the exposure period, the potential for the development of cardiovascular dependence and associated morbidity and mortality could not be evaluated.

The only effect observed in dogs during the 12 months of treatment (Ellis et al., 1984) was occasional minimal methemoglobinemia, usually less than 3% (values for control dogs were not provided). The incidence of methemoglobinemia was reported to be dose-related and was apparently present to some extent in all treated groups (data not shown). No adverse effects were observed in dogs regarding body weights, clinical chemistry parameters and gross or microscopic appearance of organs and tissues. The dose level of 25 mg nitroglycerin/kg-day can be considered a minimal effective level for methemoglobineamia for dogs in a long-term oral study.

In rats, body weight gain and final body weight were significantly reduced in the high-dose groups (Ellis et al., 1984). Significantly reduced food consumption in high-dose rats of both sexes was apparent during the first 3 weeks of the study and in females throughout the study. Unscheduled deaths occurred in all groups and were mainly due to pituitary adenomas, ulcerated subcutaneous tumors and other causes not specified. High-dose females survived significantly longer than control females, which could possibly be attributed to reduced incidence of mammary and pituitary tumors in the high dose group compared to other exposure groups. Changes in clinical chemistry and hematology parameters, monitored throughout the study, were restricted to the high-dose groups and included methemoglobinemia (10-30%, normal is <1%) and compensatory reticulocytosis, increased erythrocyte count, hematocrit and hemoglobin indicated hemoconcentration. In addition, high-dose males had elevated serum ALT, AST and alkaline phosphatase levels, suggesting hepatocellular damage and cholestasis.

Histopathologic effects were evident after 12 months of feeding in high-dose rats and consisted of increased absolute and relative liver weight, cholangiofibrosis, proliferation of the bile ducts and increased pigmentation of the spleen and kidney epithelium. Lesions observed in rats killed after 24 months of treatment were similar to those seen at 12 months, but with higher incidence and severity. Further details regarding other tissues were not provided. For rats, the high dose of 363 mg/kg-day is considered a LOAEL for hematological and hepatic effects and 31.5 mg/kg-day a NOAEL in this chronic study.

In mice, food consumption in the high-dose groups was reduced during the first week, but subsequently recovered (Ellis et al., 1984). Nevertheless, body weight in the high-dose groups was reduced throughout the study. Mortality did not differ significantly between control and treated groups of mice. After 12 months, high-dose mice had a compensated anemia (normal erythrocyte count, elevated reticulocyte count and Heinz bodies); high-dose males also had methemoglobinemia. The only nitroglycerin-related alteration seen in organs and tissues was hyperpigmentation in the liver, spleen and kidney in most high-dose mice and in some mid-dose mice. Lesions observed after 24 months of treatment were similar to those observed after 12 months; further details were not provided. The dose of 1022 mg/kg-day is considered a LOAEL for hematological effects in mice and the dose of 96.4 mg/kg-day is a NOAEL.

Ellis et al. (1984) also conducted a 13-week subchronic toxicity study in dogs, rats and mice. Beagle dogs (6/sex/dose level) were administered nitroglycerin in capsules to provide time weighted average (TWA) doses of 0, 0.04, 0.4 or 4 mg nitroglycerin/kg-day over a period of 13 weeks. Male CD rats (6/dose level) received TWA dietary doses of 0, 1.6, 17.3 or 164 mg nitroglycerin/kg-day, whereas females (6/dose level) were given 0, 1.9, 18.7 or 167 mg nitroglycerin/kg-day. Male CD-1 mice (6/dose level) were treated with 0, 5.2, 49 or 476 mg nitroglycerin/kg-day TWA, and females with 0, 5.6, 47.6 or 453 mg nitroglycerin/kg-day. Blood samples were taken from the dogs at several intervals during the study and from rats and mice at termination. All major organs and tissues of the three species were examined for gross lesions and processed for light microscopy. No adverse effects were observed in dogs. The only treatment-related effect observed in rats was reduced body weight gain (statistical significance not provided), and this was observed only in high-dose groups. No adverse effects were observed in mice other than mild to moderate extramedullary hematopoiesis in the liver and/or spleen; however, the incidence and severity of the lesion was not dose-related.

As part of the same study, Ellis et al. (1984) fed CD rats (3/sex/group) a diet containing 0 or 2.5% nitroglycerin for 13 weeks. This diet provided doses (estimated by the investigators) of 1406 mg nitroglycerin/kg-day for males and 1416 mg nitroglycerin/kg-day for females. A significant reduction in food consumption and body weight was observed in treated rats during the first 8 weeks; this resulted in a significant reduction in final body weight. Treatment with nitroglycerin induced significant increases in erythrocytes, reticulocytes, hematocrit, hemoglobin concentration and alkaline phosphatase and a decrease in fasting blood glucose; no methemoglobin was detected. Histopathological observations revealed the presence of pigment deposits in the liver and spleen and moderate to severe testicular degeneration and/or atrophy with severe-to-complete aspermatogenesis. No other effects were reported.

In 4-week short-term studies (Ellis et al., 1984), five consecutive doses of 25, 50, 100 or 200 mg nitroglycerin/kg-day, given to dogs (6/sex/dose), produced transient dose-related increases in methemoglobin. Serial determinations of methemoglobin for 24 hours after each

dose showed that the duration of methemoglobinemia was also dose-related. No other endpoints were examined.

Earlier studies in rats and mice found no effects of chronic exposure to nitroglycerin. Groups of 50 male and 48 female 8-week-old Sprague Dawley rats were given drinking water containing 0.03% nitroglycerin (31 mg/kg-day, as estimated by the researchers) for 10 months, followed by plain tap water for an additional 8 months, at which time survivors were sacrificed for necropsy, hematology and serum chemistry (5/sex), organ weight measurements and histopathological examination of a wide range of tissues (Takayama, 1975). A control group of 53 male and 49 female rats provided with plain tap water for the entire 18-month experimental period was similarly examined at termination. Mortality due to pneumonia claimed 38 male and 23 female rats in the treated group and 36 male and 32 female control rats during the experiment. Treatment had no effect on survival, behavior, general physical appearance or body weight. There were no treatment-related effects on hematological parameters or on several serum chemistry tests to detect impaired function of, or damage to, the liver and kidney; methemoglobinemia was not evaluated. There were no effects on organ weights in the treated or control rats surviving to 18 months. Treatment with nitroglycerin had no effect on incidence of non-neoplastic lesions or tumors. The tested dose of 31 mg/kg-day is a NOAEL in this study, but interpretation of the study is limited by high mortality in all groups, failure to include additional dose levels and the long recovery period between the end of treatment and toxicology evaluation. Although the treatment-free recovery period would allow for observations regarding the potential for nitroglycerin-induced dependence and rebound vasoconstriction, this was not evaluated.

Suzuki et al. (1975) provided 4-week-old C57BL/6Jms mice with drinking water in which was dissolved 0, 10, 40 or 330 mg/L nitroglycerin in saline (doses of 0, 1.5, 6.2 and 58.1 mg/kg-day estimated by the researchers). The high-dose mice (49 males and 45 females) received nitroglycerin for 52 weeks followed by control drinking water for an additional 28 weeks. The mid-dose (66 males and 49 females), low-dose (54 males and 50 females) and control (60 male and 63 females) groups received appropriate concentrations of nitroglycerin for the entire 80-week experimental period. The mice were subjected to necropsy and histopathological examination of heart, liver, kidney, spleen, bone marrow and all gross lesions. There were no clearly treatment-related effects on behavior or general appearance. The body weights of high-dose mice of both sexes were below their respective controls starting at about 13-14 months, but statistical analyses were not performed and the difference from controls was less than 10%. The investigators noted that a higher incidence of early mortality (30-40 weeks of treatment) occurred in the high-dose females compared with controls, but the effect appeared to be slight and statistical analysis was not performed. Histopathologic examination revealed no treatment related noncarcinogenic effects. The high dose of 58.1 mg/kg-day is a NOAEL in this study. The study was limited by the long recovery period between the end of treatment and toxicology evaluation in the high-dose group and failure to find an effect level. Although the treatment-free recovery period would allow for observations regarding the potential for nitroglycerin-induced dependence and rebound vasoconstriction, this was not evaluated.

Wang and Fung (2002) measured arterial pressure in exposed obese and normal SD rats (1 and 10 µg/min) challenged hourly with 30 µg nitroglycerin via intravenous route. They neither observed any development of nitroglycerin tolerance or changes in hemodynamic effects.

Information on the developmental and reproductive toxicity of nitroglycerin is limited to an oral 3-generation reproduction study in animals (Ellis et al., 1978, as discussed in U.S. EPA, 1987) and several studies involving parenteral administration of nitroglycerin (Oketani et al., 1981a,b,c,d; Yallampalli and Garfield, 1993). In the 3-generation reproduction study (Ellis et al., 1978), groups of male and female rats were fed diets containing 0, 0.01, 0.1 or 1.0% nitroglycerin (the authors estimated the doses to be 0, 3.04, 31.5 and 363 mg/kg-day for males and 0, 3.99, 31.5 and 434 mg/kg-day for females). For the parental generation, groups of 10 male and 20 female rats were exposed to nitroglycerin for 6 months prior to mating and during gestation and lactation of two litters. Groups of F_{1b} rats (10-12 pairs) were fed the nitroglycerin diet during growth, mating, gestation and lactation. Groups of F_{2b} rats were similarly treated. No effects on fertility were observed in the F_0 rats. Severely impaired fertility was observed in the high-dose F_1 groups, attributed to aspermatogenesis with increased interstitial tissue in the testes and significantly smaller testicular size in the high-dose males. Litter size, live-born index, birth weight, viability and lactation indices were decreased in the F_{1a}, F_{1b} and F_{2a} offspring of the high-dose group. The authors attributed these effects to a marked decrease in food intake (65% lower than controls for the F_0 females) and body weight (81% lower than controls for the F_0 females) in the dams. The marked reduction in food consumption may be attributed to taste aversion; thus contributing to smaller testicular size and impaired fertility.

Oketani et al. (1981a,b) observed no adverse developmental effects (fetal and postnatal development, fertility of offspring, incidences of external, visceral or skeletal abnormalities) in the offspring of rats treated intraperitoneally with doses of 1, 10 or 20 mg/kg-day nitroglycerin on gestation days 7-17 (Oketani et al., 1981a) or on gestation day 17 through lactation day 21 (Oketani et al., 1981b). The authors noted that a transient convulsion or sedation was occasionally observed in the dams treated with 20 mg/kg-day nitroglycerin. No developmental effects (pup mortality or pup size) were reported in rats administered 240 or 480 µg/day nitroglycerin via an osmotic pump (0.8 or 1.5 mg/kg-day using the initial body weight of 0.312 kg) on gestation days 17-22 (Yallampalli and Garfield, 1993). No developmental effects were observed in rabbits intravenously administered 0.5, 1 or 4 mg/kg-day nitroglycerin on gestation days 6-18. Transient convulsions were observed in the does treated with 4 mg/kg-day (Oketani et al., 1981c).

In a reproduction study conducted by Oketani et al. (1981d), groups of male and female rats received intraperitoneal doses of 1, 10 or 20 mg/kg-day nitroglycerin. The male rats were dosed for 63 days prior to mating and the female rats were dosed for 14 days prior to mating and during gestation days 0-7. A transient convulsion or sedation was occasionally observed in rats administered 20 mg/kg-day. No adverse effects on mating, fertility or reproductive indices (no details provided) were observed. In addition, no developmental effects were observed in the offspring.

DERIVATION OF A PROVISIONAL RfD FOR NITROGLYCERIN

It is generally accepted that the adverse effects associated with therapeutic and occupational exposure to nitroglycerin are secondary to actions on the cardiovascular system, with vasodilation as the initiating event (Kerins et al., 2001). Therefore, vasodilation is considered as the critical effect upon which to base the RfD. Adverse effects of concern from nitroglycerin for all routes of exposure include hypotension, reflex tachycardia, cardiac

arrhythmias, headache, dizziness, nausea and vomiting. Effects can range in intensity from mild to severe, depending upon dose and individual susceptibility (Kerins et al., 2001). An additional concern associated with nitroglycerin-induced vasodilation is the development of organic nitrate dependence, whereby constant exposure is required to maintain optimum cardiovascular function. Upon rapid withdrawal from exposure in adapted individuals, severe rebound hypertension leading to heart attack and stroke can result, as reported in munitions workers (Daum, 1983). Dependence to organic nitrates can develop within days of exposure (Katzung and Chatterjee, 2001; Kerins et al., 2001) but may also develop following a more prolonged exposure duration (Daum, 1983). At exposure levels below those causing acute vasodilation, acute adverse effects (e.g., headache, hypotension) and dependence are not expected to develop. Furthermore, due to rapid elimination of nitroglycerin and the active dinitroglycerol metabolites, bioaccumulation is not expected in long-term/chronic exposure, thus eliminating the possibility of cumulative damage to the target organ(s).

Due to their rapid elimination (2-9 minutes for nitroglycerin and 40 minutes to 3 hours for the dinitroglycerol metabolites), nitroglycerin and the dinitroglycerol metabolites do not accumulate in the body. In practical terms, this means that the adverse effect level (for vasodilatory-related effects) for subchronic and chronic exposure will be no lower than the adverse effect level for acute exposure. For this reason, a subchronic and chronic p-RfD can be derived based on the acute dose-response information. Therefore, a p-RfD based on acute vasodilation is applicable to subchronic and chronic exposures.

A substantial amount of information exists regarding the effects of nitroglycerin in humans due to its therapeutic use and in the manufacturing of explosives. Since nitroglycerin has a short half-life (3-9 minutes) and quantitative data for continuous occupational exposure in epidemiological studies are inadequate, these studies were not used as a basis for the derivation of toxicity values. In general, these studies lack data regarding exposure levels and duration of exposure as well as information on confounding factors such as health history and life style of the subjects. Simultaneous exposure to other chemicals, as in the case of workers in the manufacturing of dynamite, is an additional confounding factor.

In clinical medicine, oral nitroglycerin is used for long-term control of angina pectoris and is only administered as sustained- or extended-release preparations designed to provide stable blood levels and therapeutic efficacy for 6-8 hours (Katzung and Chatterjee, 2001). The lowest prescribed single oral dose of sustained-release nitroglycerin is 2.5 mg (equivalent to 0.036 mg/kg-day, assuming a typical body weight of 70 kg) with a recommended maximum total daily oral dose of 38 mg/day (Katzung and Chatterjee, 2001; Kerins et al., 2001). Therapeutic exposure to oral sustained-release nitroglycerin is similar to the conditions of occupational exposure, in which daily exposure occurs over the course of the workday. When administered by the sublingual route, lower doses of nitroglycerin (0.15-1.2 mg) are effective in controlling acute angina (Katzung and Chatterjee, 2001). However, due to differences in the pharmacokinetics of nitroglycerin administered by the oral and sublingual routes (e.g., no first pass metabolism following sublingual administration), sublingual exposure is not considered relevant to the quantitative development of an RfD for oral exposure.

The lowest prescribed therapeutic oral dose of 0.036 mg/kg-day is associated with adverse effects caused by vasodilation (Katzung and Chatterjee, 2001; Kerins et al., 2001). Since no information on the dose-response relationship for oral nitroglycerin was identified in

the published literature, the RfD is based on the lowest prescribed oral dose of 0.036 mg/kg-day, which is considered the LOAEL for vasodilation. No evidence is available from the clinical literature to suggest that chronic exposure to therapeutic doses of nitroglycerin by any route of administration results in toxicity to the liver or other organs, as shown in subchronic and chronic exposure studies in laboratory animals (Ellis et al., 1984). It is possible that toxicity to other organs (liver) in humans may occur at higher doses than those which produce vasodilation, which is consistent with the relatively high NOAELs and LOAELs for hepatic toxicity in animals. However, due to the severity of acute adverse effects associated with vasodilation, clinical studies in humans have not explored the potential adverse effects of high-dose nitroglycerin in humans. Dosages necessary to cause methemoglobinemia observed in occupational studies are much greater than those causing vasodilation (Katzung and Chatterjee, 2001). Furthermore, tolerance dosage, which could trigger angina or a myocardial infarction in a sensitive individual with cardiovascular disease, appears to be higher. Thus, an RfD based on the lowest prescribed oral therapeutic dose would be considered protective for the development of methemoglobinemia.

The NOAEL of 31.5 mg nitroglycerin/kg-day for methemoglobinemia and liver effects from the chronic oral study conducted by Ellis et al. (1984) in rats was not considered as a basis for the RfD since vasodilatory-related adverse effects in humans occur at doses approximately 1000 times lower than the rat NOAEL. The available animal studies did not examine vasodilation as an endpoint, so it is possible that vasodilation-related adverse effects occurred at lower exposure levels than methemoglobinemia and liver effects.

The subchronic and chronic p-RfD for nitroglycerin was derived from the LOAEL of 0.036 mg/kg-day for acute adverse effects related to vasodilation as follows:

$$\begin{aligned} \text{p-RfD} &= \text{LOAEL} \div \text{UF} \\ &= 0.036 \text{ mg/kg-day} \div 300 \\ &= 1\text{E-}4 \text{ mg/kg-day } (0.12 \text{ µg/kg-day}) \end{aligned}$$

Dividing the LOAEL of 0.036 mg/kg-day by an uncertainty factor of 300 yields a **p-RfD of 1E-4 mg/kg-day** (0.12 µg/kg-day). The uncertainty factor of 300 is composed of factors of 10 for use of a LOAEL for potentially serious adverse effects, 3 for incomplete database (absence of dose-response data) and 10 to account for human variability, including sensitive subgroups (individuals with cardiovascular diseases such as hypertension, angina, congestive heart failure, children with inadequate MFO or metabolic activity and cardiac arrhythmias). Although this p-RfD is based on the acute vasodilatory effects of nitroglycerin, adverse effects related to vasodilation will not occur over the course of subchronic and chronic exposures. Thus, the p-RfD based on acute effects is considered protective for subchronic and chronic exposures to nitroglycerin.

Confidence in the LOAEL value for vasodilation is low-to-medium. The LOAEL value for vasodilation in humans is based on the lowest prescribed oral dose used for the therapeutic control of angina pectoris in patients. Dose-response data for oral nitroglycerin were not available. It is possible that vasodilation leading to adverse effects in patients and healthy individuals may occur at lower oral exposure levels. Although organic nitrates have been used for over 100 years in the treatment of cardiovascular diseases and clinical experience does not suggest that significant adverse effects other than those related to vasodilation will results from

chronic exposure, controlled studies evaluating the effects of chronic exposure in humans have not been published. Low-to-medium confidence in the provisional RfD results.

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4-19-05

Provisional Peer Reviewed Toxicity Values for

Nitroglycerin
(CASRN 55-63-0)

Derivation of Subchronic and Chronic Inhalation RfCs

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	**provisional inhalation unit risk**
p-OSF	**provisional oral slope factor**
p-RfC	**provisional inhalation reference concentration**
p-RfD	**provisional oral reference dose**
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	**Provisional Peer Reviewed Toxicity Value**
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR NITROGLYCERIN (CASRN 55-63-0)
Derivation of Subchronic and Chronic Inhalation RfCs

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

An RfC for nitroglycerin (also known as trinitroglycerol) is not available on IRIS (U.S. EPA, 2003) or in the HEAST (U.S. EPA, 1997). The CARA list (U.S. EPA, 1991, 1994) does not include any other review documents for nitroglycerin, but a Drinking Water Health Advisory document is available (U.S. EPA, 1987). Lee et al. (1977), on a contract with the U.S. Army Medical Bioengineering Research Laboratory (U.S. AMBRDL), developed a report on a mammalian toxicity study on Trinitroglycerin. The U.S. Army Center for Health Promotion and Preventative Medicine (U.S. ACHPPM) completed a wildlife toxicity assessment for Nitroglycerin (U.S. ACHPPM, 2000). Neither ATSDR (2003), NTP (2003), IARC (2003), nor WHO (2003) have produced documents regarding nitroglycerin. ACGIH (2001, 2003) has adopted a threshold limit value - time weighted average (TLV-TWA) of 0.05 ppm (0.46 mg/m^3) for nitroglycerin to minimize the potential for vasodilation expressed by headache and decreases

in blood pressure. The TLV-TWA is based on human data and by analogy to propylene glycol dinitrate. NIOSH (2003) recommends a short-term exposure level (STEL) of 0.1 mg/m^3 and an immediately dangerous to life and health (IDLH) of 75 mg/m^3. The OSHA (2003) permissible exposure limit (PEL) is 0.2 ppm as a ceiling limit. Literature searches were conducted for the period from 1965 to July 2003 in the following databases: TOXLINE (including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS, and TSCATS. Additional literature searches from July 2003 through September 2004 were conducted by NCEA-Cincinnati using MEDLINE, TOXLINE, Chemical and Biological Abstracts databases.

REVIEW OF PERTINENT LITERATURE

Human Studies

Contact with nitroglycerin can occur through occupational exposure of persons employed in the explosives industry, or through its medical use in the relief of angina pectoris. Among the key issues to be weighed in considering the extent of nitroglycerin's toxicity in exposed individuals are (1) the development of adverse symptoms such as headache, dizziness, palpitations, and nausea in persons exposed to nitroglycerin therapeutically or (along with other compounds) in the workplace; (2) the development of tolerance to the pharmacological effects of nitroglycerin, whereby the severity of side-effects of the compound can become reduced, or where an increase in the administered dose becomes necessary to maintain stability; (3) the association of chronic exposure to the compound with organic nitrate dependence, in which withdrawal from the treatment can cause (occasionally fatal) heart attack and stroke in subjects who may have adapted to the continuous presence of the compound to maintain an optimum level of coronary flow; (4) the consequent desirability of maintaining stable levels of organic nitrates and their derivatives in patients to guard against debilitating fluctuations in body burden; and (5) the potential for the compound to be associated with systemic toxicity, as indicated by long-term exposure studies in laboratory animals.

The capacity of aliphatic nitrates such as nitroglycerin to lower blood pressure forms the basis for the compound's use in medicine; for example, in the control of angina pectoris (NIOSH, 1978). As a drug, nitroglycerin is prescribed for intermittent use, with dose levels established by the degree of relief from symptoms afforded to the patient as the nitroglycerin amount is raised. This approach reflects the rapid onset of the compound's pharmacological effects and the marked individuality of response to treatment among different patients (Abrams, 1980). Where the requirement for organic nitrate therapy becomes repeated, well-recognized side-effects of nitroglycerin treatment such as headache, palpitations, nausea, and vomiting may become reduced as the compound becomes tolerated by the host (Abrams, 1980). Unfortunately, nitroglycerin has the capacity to cause dependence, whereby cessation of repeated exposure to

the compound may itself become associated with sometimes severe reactions, such as angina, weakness, palpitations, difficulty in breathing, and, in some cases, death (Daum, 1983). This is an especially important issue in occupationally exposed workers, such as those in the explosives industry, whose normally elevated organic nitrate body burden becomes depleted during a period of absence from the workplace, such as the weekend. The well-documented phenomenon of "Monday Morning Death" has been described for munitions workers in whom cardiovascular events can be triggered by unrestrained compensatory vasoconstriction, as the organic nitrate in the body becomes reduced (Abrams, 1980). Incidence of sudden death in such persons is up to 15 times the expected rate (Daum, 1983). Kristensen (1989) discussed an apparent double effect of nitroglycerin in cardiovascular disease, where the incidence of "Monday Morning Death" was compounded by an apparently increased risk of heart attack and stroke that continued in chronically exposed subjects long after the cessation of exposure.

Craig et al. (1985) carried out a death certificate study of persons who had been employed at an explosives factory in Scotland on January 1, 1965. The investigation covered the time period 1965 to 1980, and compared the causes of death of potentially exposed workers with internal controls (unexposed workers at the same factory) and members of the general male population. Blasting workers (exposed to nitroglycerin and ethylene glycol dinitrate) and propellant workers (exposed to nitroglycerin alone) were subdivided by age and then by high- and low-exposure subcategories, depending on the position of their work station or the nature of their duties. Causes of death for each target group included acute myocardial infarction (MI), ischemic heart disease (IHD), cerebrovascular disease, lung cancer, cancer of the colon or rectum, and all cancers. Among the non-cancer related responses, there was an apparent increased incidence of MI and IHD in the 15-49 age group of high-exposure blasting workers compared with internal controls or with members of the general population. However, these findings do not necessarily implicate nitroglycerin, as no effect was reported among propellant workers exposed to nitroglycerin alone.

Animal Studies

No studies were located regarding inhalation exposure of animals to nitroglycerin.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR NITROGLYCERIN

As previously indicated, a substantial amount of information exists regarding the effects of nitroglycerin in humans, mainly due to its use as a pharmaceutical and in the manufacturing of explosives. However, lack of quantitative data in epidemiological studies preclude their use as basis for derivation of risk assessment values. In general, these studies lack data regarding exposure levels and duration of exposure, as well as information on confounding factors such as

health history and life style of the subjects. Simultaneous exposure to other chemicals, as in the case of workers in the manufacturing of dynamite, is an additional problem.

The lack of adequate epidemiological studies in humans, or subchronic or chronic inhalation toxicity data in animals precludes derivation of a subchronic or chronic p-RfC for nitroglycerin.

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9-13-2005

Provisional Peer Reviewed Toxicity Values for

Nitroglycerin
(CASRN 55-63-0)

Derivation of a Carcinogenicity Assessment

Superfund Health Risk Technical Support Center
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	**provisional inhalation unit risk**
p-OSF	**provisional oral slope factor**
p-RfC	**provisional inhalation reference concentration**
p-RfD	**provisional oral reference dose**
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	**Provisional Peer Reviewed Toxicity Value**
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

9-13-2005

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR NITROGLYCERIN (CASRN 55-63-0)
Derivation of a Carcinogenicity Assessment

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

A cancer assessment for nitroglycerin (also known as trinitroglycerol) is not available on IRIS (U.S. EPA, 2005a) or in the HEAST (U.S. EPA, 1997). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2002) includes a cancer risk for nitroglycerin based on U.S. EPA (1987) analysis of liver tumors in a lifetime study in rats (Ellis et al., 1984). The CARA list (U.S. EPA, 1991, 1994) does not include any other review documents for nitroglycerin. Lee et al. (1977), on a contract with the U.S. Army Medical Bioengineering Research Laboratory (U.S. AMBRDL), developed a report on a mammalian toxicity study on Trinitroglycerin. The U.S. Army Center for Health Promotion and Preventative Medicine (U.S. ACHPPM) completed a wildlife toxicity assessment for Nitroglycerin (U.S. ACHPPM, 2000). Neither ATSDR (2003), NTP (2003), IARC (2003), nor WHO (2003) have produced documents regarding nitroglycerin. Literature searches were conducted for the period from 1965 to July

2003 in the following databases: TOXLINE (including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS, and TSCATS. Additional literature searches from July 2003 through September 2004 were conducted by NCEA-Cincinnati using MEDLINE, TOXLINE, Chemical and Biological Abstracts databases.

REVIEW OF PERTINENT LITERATURE

Human Studies

Craig et al. (1985) investigated the cause of death, taken from death certificates, of male workers employed in a large explosives factory in Scotland on January 1, 1965 and followed for 16 years. The workers were divided into those exposed to nitroglycerin alone (propellant workers, n=224), to nitroglycerin and ethylene glycol dinitrate in an approximate ratio of 4:1 (blasting workers, n=659), or to neither compound (n=3159). The exposed workers were subdivided into high- and low-exposure categories, but no attempt was made to quantify exposures. The workers were further subdivided into those 15-49 or 50-64 years of age on January 1, 1965. The numbers of deaths from all cancers, lung cancer, and colorectal cancer among the exposed workers were compared to expected numbers based on the unexposed workers (internal controls) and on the general male population of the County of Ayr in which 98% of the workers had resided. None of the categories or subcategories of exposed workers had significantly increased occurrence of death due to all cancers, lung cancer, or colorectal cancer, compared with either internal controls or the general male population of the County of Ayr, with the following exception. The high-exposure category of blasting workers aged 15-49 had a significant excess of lung cancer when compared with internal controls, but not when compared with the general male population of the County of Ayr. Because the blasting workers were also exposed to ethylene glycol dinitrate, and because a significant excess of lung cancer was not observed in workers exposed to nitroglycerin alone, the observation of excess lung cancer in the high exposure blasting workers does not support a causal relationship between exposure to nitroglycerin and lung cancer.

Animal Studies

In a chronic toxicity study conducted by Ellis et al. (1984), groups of 6 male and 6 female beagle dogs were administered daily capsules containing 0, 1, 5, or 25 mg/kg-day nitroglycerin for 12 months, and groups of CD rats (38/sex/group) and CD-1 mice (58/sex/group) were fed diets containing 0, 0.01, 0.1, or 1% nitroglycerin for 24 months (the authors estimated that the dietary concentrations corresponded to doses of 0, 3.04, 31.5, and 363 mg/kg-day for male rats; 0, 3.99, 38.1, and 434 mg/kg-day for female rats; 0, 11.1, 114.6, and 1022 mg/kg-day for male mice; and 0, 9.72, 96.4, and 1058 mg/kg-day for female mice). Groups of rats and mice

(8/sex/species/group) were killed after 12 months of exposure. The only effect observed in the dogs was an occasional, but dose-related, increase in blood methemoglobin concentration. There was no mention of any tumors in the dogs, but the scope of the gross and histopathologic examination is unclear. In addition, the duration of exposure was probably insufficient to induce tumors with a long latency, and it is doubtful that the maximum tolerated dose was reached. This study is considered inadequate for evaluating the carcinogenic potential of nitroglycerin in dogs.

Rats of both sexes fed the 1% diet consumed significantly less food than controls during the first three weeks of the study; females consumed significantly less food throughout the experiment. The body weights of the rats fed the 1% diet were notably less than the controls throughout the experiment, and these rats had considerably less body fat (particularly abdominal fat) than controls. Treatment with the 0.01 or 0.1% diets had no significant effect on food consumption or body weight, although rats fed the 0.1% diet consumed slightly less and weighed slightly less than controls, particularly after three months (males) and 12 months (females) of exposure. Treatment had no adverse effect on survival; females fed the 1% diet had significantly longer survival than female control rats, presumably because of a lower incidence of pituitary and mammary tumors. Rats fed the 1% diet also had methemoglobinemia and serum biochemical and histopathologic evidence of liver damage (Ellis et al., 1984). On the basis of the effects on food consumption and body weight, methemoglobinemia and evidence of liver damage, it is judged that the maximum tolerated dietary dose was reached in the rats. Evidence of nitroglycerin-induced hepatocellular carcinogenicity in rats was apparent at the interim (12 month) sacrifice. Preneoplastic lesions occurred in the livers of 1/8, 4/6, 6/7 and 6/8 males and of 1/8, 2/7, 3/8 and 8/8 females fed the 0, 0.01, 0.1 and 1% diets, respectively. The authors noted a dose-related increase in both the incidence and severity of these preneoplastic lesions. Neoplastic nodules or hepatocellular carcinomas occurred in one male fed the 0.1% diet and in four males and one female fed the 1% diet (Ellis et al., 1984).

At the termination of the experiment, the combined incidence of neoplastic nodules and hepatocellular carcinomas was significantly increased in rats of both sexes fed the 1% diet (**Table 1**). The incidence of testicular interstitial cell tumors was significantly higher in rats fed the 1% diet than in the controls. The incidence of pituitary chromophobe adenomas in rats of both sexes fed the 1% diet and the incidence of mammary gland tumors in females fed the 1% diet was notably reduced compared with the appropriate controls (Ellis et al., 1984).

Mice of both sexes fed the 1% nitroglycerin diet had sharply reduced food consumption and frank weight loss during the first week of treatment. Thereafter, these mice began gaining weight, but their body weights remained below the controls throughout the experiment. Mice of both sexes fed the 1% diet evaluated at 12 months of treatment had a compensated anemia and erythrocytes containing Heinz bodies, and the males had a significant ($p<0.05$), but low-grade methemoglobinemia. Deposits of hemoglobin-derived pigments were observed at the 12-month

Table 1. Incidence of tumors in CD rats fed diets containing nitroglycerin for 24 months*

Sex	Tumor Type	Dietary level (percent)	Dose mg/kg-day	Incidence[a] (p value)[b]
Male	Hepatocellular carcinoma or neoplastic nodule	0	0	1/24 (<0.001)
		0.01	3.04	0/28 (NS)
		0.1	31.5	4/26 (NS)
		1	363	15/21 (<0.001)
Female	Hepatocellular carcinoma or neoplastic nodule	0	0	0/29 (<0.001)
		0.01	3.99	1/32 (NS)
		0.1	38.1	3/28 (NS)
		1	434	16/25 (<0.001)
Male	Testicular interstitial cell tumor	0	0	2/24 (<0.001)
		0.01	3.04	1/28 (NS)
		0.1	31.5	3/26 (NS)
		1	363	11/21 (0.001)

* Source: Ellis et al., 1984

[a] number of lesion-bearing rats/number of rats with readable slides

[b] p Values presented with the control incidence refer to the Cochran-Armitage trend test; p values presented with the treated group incidence refer to the Fisher exact test comparing the treated and control groups; all statistical analyses performed by SRC.

NS = not statistically significant

sacrifice and at termination in the liver, spleen and kidney of most mice fed the 1% diet and some (not otherwise specified) mice fed the 0.1% diet (Ellis et al., 1984). On the basis of the effects on food consumption and body weight, the compensated anemia, methemoglobinemia and the deposits of pigments in the tissues, it is judged that the maximum tolerated dietary dose had been reached in the mice. There was no evidence of carcinogenicity in any tissues of the mice (Ellis et al., 1984), including the pituitary gland (Hong, 1991), which was identified as a potential site of nitroglycerin carcinogenicity in an earlier study (Suzuki et al., 1975, described below).

A study by Tamano et al. (1996) provided corroborating evidence that nitroglycerin can produce liver tumors in rats. Male F344/N rats received nitroglycerin, either as a single corn oil bolus of 1200 mg/kg via gavage (n=80) at 6 weeks of age, or added to the feed at a concentration of 1% (≈500 mg/kg-day, assuming a rat consumes 5% of its body weight per day) for up to 78 weeks starting at 8 weeks of age (n=30), or both (n=80). Some animals in the bolus-only group and those that received both treatments were subjected to 2/3 partial hepatectomy at 9 weeks of age. An untreated control group included 10 animals. Interim sacrifices of varying numbers of

animals among the groups were carried out at 14, 32, 52, 78 and 84 weeks of age. These animals and others dying prematurely were subjected to necropsy, with the livers weighed and examined for gross lesions. Pieces of liver, especially those with visible lesions, were examined histopathologically. DNA from liver tumors was extracted and examined for mutations in the coding regions of the K-ras (exons 1 and 2) and p53 (exons 5 to 8) genes. Body weight gain was reduced in the rats receiving nitroglycerin in the diet compared with those animals receiving the compound via gavage, or to controls. Marked morphological liver lesions became evident in the diet-exposed groups from 32 weeks of age. These included tan-colored foci, with later appearance of nodules and masses. At 78 weeks of age, both groups of rats receiving the compound via the diet displayed >50% incidence of combined hepatocellular adenomas and carcinomas. No liver tumors were found in control rats or those exposed only by single bolus. DNA examination of 18 liver tumors from diet-treated rats found no mutations in the p53 gene, but K-ras point mutations in 8 tumors (primarily those with cholangiocellular elements). All the mutations were in codon 12 of exon 1 (4 GGT-GTT transversions, 3 GGT-GAT transitions, and 1 GGT-TGT transversion). The authors considered their data to represent only marginal evidence of a gene modification as the initiating event in nitroglycerin-induced carcinogenicity.

An earlier study in rats found no evidence of carcinogenicity, but included only a single, relatively low dose-level. A group of 50 male and 48 female 8-week-old Sprague Dawley rats were given drinking water containing 0.03% nitroglycerin (31 mg/kg-day, as estimated by the researchers) for 10 months, followed by plain tap water for an additional 8 months, at which time survivors were sacrificed for necropsy, hematology and serum chemistry (5 rats per sex), organ weight measurements and histopathological examination of a wide range of tissues (Takayama, 1975). A control group of 53 male and 49 female rats, provided with plain tap water for the entire 18-month experimental period, was similarly examined at termination. Mortality due to pneumonia claimed 38 male and 23 female rats in the treated group and 36 male and 32 female control rats during the experiment. Treatment had no effect on survival, behavior, general physical appearance, or body weight. There were no treatment-related effects on hematological parameters, or on several serum chemistry tests to detect impaired function of, or damage to, the liver and kidney. Methemoglobinemia was not evaluated. There were no effects on organ weights in the treated or control rats surviving to 18 months. Treatment with nitroglycerin had no effect on incidence of nonneoplastic lesions or tumors. The only tumors observed were mammary gland tumors in 8/44 treated female rats and 5/45 female controls and a pituitary adenoma in one control female. This study found no evidence of carcinogenicity in rats, but is not an adequate study due to use of a single low exposure level that did not approach the maximum tolerated dose, high non-treatment-related mortality, and short exposure duration.

Marginal evidence for carcinogenicity was found in an early study in mice. Suzuki et al. (1975) provided 4-week-old C57BL/6Jms mice with drinking water in which was dissolved 0, 10, 40 or 330 mg/L nitroglycerin in saline (doses of 0, 1.5, 6.2, and 58.1 mg/kg-day estimated by the researchers). The high-dose mice (49 males and 45 females) received nitroglycerin for 52

weeks followed by control drinking water for an additional 28 weeks. The mid-dose (66 males and 49 females), low-dose (54 males and 50 females), and control (60 male and 63 females) groups received appropriate concentrations of nitroglycerin for the entire 80-week experimental period. The mice were subjected to necropsy and histopathological examination of heart, liver, kidney, spleen, bone marrow, and all gross lesions. There were no clearly treatment-related effects on behavior or general appearance. The body weights of high-dose mice of both sexes were below their respective controls starting at about 13-14 months, but statistical analyses were not performed and the difference from controls was less than 10%. The investigators noted that a higher incidence of early mortality (30-40 weeks of treatment) occurred in the high-dose females compared with controls, but the effect appeared to be slight and statistical analysis was not performed. Histopathologic examination revealed no treatment related noncarcinogenic effects. It is doubtful that the maximum tolerated dose was attained in this experiment. Tumor incidence was generally comparable to controls, except that histopathologic examination revealed pituitary adenomas in 1/50, 1/40, 3/39 and 6/34 females in the control, low-, mid- and high-dose groups, respectively. The incidence in the high-dose females was significantly higher than in control females ($p<0.05$, Fisher exact test performed at SRC) and the Cochran-Armitage test for trend was positive ($p<0.01$, performed at SRC). This study provides some evidence for carcinogenicity of nitroglycerin in female mice, but is limited by apparent failure to attain the maximum tolerated dose, relatively short exposure duration (especially of the high-dose group), lack of statistical analysis, and failure of the researchers to discuss the increase in pituitary tumors.

Mode of Action

Studies of the genotoxicity of nitroglycerin have produced mixed results. Simmon et al. (1977) found that nitroglycerin did not produce mutations in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100 with or without metabolic activation or in *Saccharomyces cerevisiae*. Other studies found mutagenic activity in *S. typhimurium* strains TA1535 and/or TA1537 with or without metabolic activity (Ellis et al., 1978a; Maragos et al., 1993). In mammalian cells, negative results were found for mutagenicity in Chinese Hamster Ovary cells without metabolic activation (Lee et al., 1976). *In vivo* studies were negative. No changes were observed in the number of tetraploids, frequency of chromatid breaks, and gaps or translocations in bone marrow and kidney cell cultures from rats fed nitroglycerin for 13 weeks or 24 months (Ellis et al., 1978b) or in chromosome aberrations in peripheral lymphocytes or kidney cultures from dogs and rats fed nitroglycerin for 4-9 weeks (Lee et al., 1976). An evaluation of the available data supports the conclusion that the data are not sufficient to determine the mutagenicity of nitroglycerin and therefore, are not sufficient to evaluate a mutagenic mode of action (MOA) of nitroglycerin as described in the 2005 revised Cancer Guidelines (U.S. EPA, 2005b).

PROVISIONAL WEIGHT-OF-EVIDENCE CLASSIFICATION

Human data are limited to the study by Craig et al. (1985) of mortality in workers in a large explosives factory in Scotland. The high-exposure group of blasting workers experienced an excess of death due to lung cancer; however, interpretation is confounded by simultaneous exposure to ethylene glycol dinitrate. Although the study found no clear association between exposure to nitroglycerin and excess deaths from all cancers, lung cancers or colorectal cancer, the study has several limitations. The investigators did not quantify exposures other than to state that the product handled by the blasting workers contained nitroglycerin and ethylene glycol dinitrate in the ratio of 4:1. They also stated that ethylene glycol dinitrate is more volatile than nitroglycerin and was more important than nitroglycerin in the exposed blasting workers. Furthermore, it is not clear that the cancer classifications studied (all cancers, lung cancer and colorectal cancer) were sufficient to detect all excess nitroglycerin-induced cancer deaths. The power of the study to detect cancer is further compromised because the exposed groups (especially the propellant workers exposed to nitroglycerin alone) were small, because the endpoint studied was death, and because the follow-up period was limited to 16 years. Therefore, the Craig et al. (1985) study is considered to be an inadequate cancer study.

Animal studies include the 12-month dog study and the 24-month rat and mouse studies by Ellis et al. (1984), the 20-month rat study by Tamano et al. (1996), the 18-month rat study by Takayama (1975) and the 80-week mouse study by Suzuki et al. (1975). As noted above, the dog study is inadequate for evaluating the potential carcinogenicity of nitroglycerin to dogs. The rat study by Ellis et al. (1984) administered appropriate doses by the most relevant route to adequately sized groups of both sexes for sufficient time to induce a compound-related carcinogenic effect (liver and testicular tumors) and provide sufficient data for quantitative risk assessment. The study by Tamano et al. (1996) provided corroborating evidence for induction by nitroglycerin of liver tumors in rats. Although Takayama (1975) did not observe a carcinogenic effect in rats exposed for 10 months, the relatively short duration of exposure and the failure to give the maximum tolerated dose render this study inadequate for evaluation of the carcinogenicity of nitroglycerin to rats.

In mice, the situation is less straightforward. There was a statistically significant (analysis at SRC) increase in pituitary adenomas in female mice treated with 58 mg/kg-day for 12 months (Suzuki et al., 1975). The study, however, was limited by use of low doses and failure to attain the maximum tolerated dose, relatively short exposure duration (especially of the high-dose group), lack of statistical analysis, and failure of the researchers to discuss the increase in pituitary tumors. A subsequent, adequately conducted study, in which mice were treated with doses as high as 1000 mg/kg-day and the maximum tolerated dose was achieved, found no evidence of a carcinogenic response in any tissue, including the pituitary gland (Ellis et al., 1984). Therefore, there is inadequate evidence for carcinogenicity of nitroglycerin in mice.

Genotoxicity studies provided some evidence for mutagenicity induced by nitroglycerin (Ellis et al., 1978a; Maragos et al., 1993), although some studies produced negative results. The Tamano et al. (1996) cancer study found marginal evidence for a gene modification (K-ras point mutations) as the initiating event in nitroglycerin-induced liver carcinogenicity.

Collectively, the data are considered to provide sufficient evidence of the carcinogenicity of nitroglycerin in animals, largely because of the highly significantly increased incidence of hepatocellular carcinomas in male and female rats and interstitial cell testicular tumors in male rats in the Ellis et al. (1984) study. This determination is supported by the early appearance (at the 12-month interim sacrifice) of the neoplastic response in the liver in the Ellis et al. (1984) study and the high incidence of liver tumors in the diet-exposed rats in the Tamano et al. (1996) study. The 1998 PPRTV document for nitroglycerin is based on essentially the same literature as the current review. The 1998 evaluation, based on the weight-of- evidence, classified nitroglycerin as *likely to be carcinogenic to humans* using the proposed 1996 guidelines (U.S. EPA, 1996), and assigned a designation of 'C' - *a possible human carcinogen* in accordance with the 1986 cancer guidelines (U.S. EPA, 1986). Compared with the internal control a significant increase in incidence of lung cancer was observed in the 15-49 age group of explosives factory workers categorized as having high nitroglycerin exposure (Craig, et al., 1985). However, this cancer incidence rate was not found to be significant when compared with the general male population of the County of Ayr, Scotland, where the explosive factory was located. A literature search revealed no cancer epidemiological studies on patients treated medically with nitroglycerin. However, a few case reports were found on cancer in patients using nitroglycerin. Based on these studies, no conclusion can be derived on whether or not therapeutic use of nitroglycerin is associated with cancer. Using the 2005 Cancer Guidelines (U.S. EPA, 2005b) nitroglycerin is classified as *likely to be carcinogenic to humans* because it has tested positive in experimental animals without evidence of carcinogenicity in humans. Data on mode of action are not conclusive.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

Provisional Oral Slope Factor

Three sets of data are available (see **Table 1**) from which it is possible to estimate a provisional oral slope factor for the carcinogenic potency of nitroglycerin: hepatocellular tumors in male and female rats and testicular interstitial cell tumors in male rats (Ellis et al., 1984). Dose-response modeling was performed for only the liver tumors because the induction of liver tumors by nitroglycerin was supported by the findings of Tamano et al. (1996).

Table 2 shows the relationship between concentrations of nitroglycerin in the feed, oral doses to the rats, human equivalent oral doses, and tumor incidences. Mean dose estimates in

Table 2. Data used for dose-response modeling for nitroglycerin*

Nitroglycerin in Feed %	Sex	Doses of Nitroglycerin		Combined hepatocellular adenoma and carcinoma tumor incidence
		mg/kg-day (rat)	mg/kg-day (human)†	
0	male	0	0	1/24
	female	0	0	0/29
0.01	male	3.04	0.96	0/28
	female	3.99	1.1	1/32
0.1	male	31.5	9.8	4/26
	female	38.1	10.5	3/28
1.0	male	363	107	15/21
	female	434	108	16/25

* Source: Ellis et al., 1984

† calculated using the cross-species scaling factor of body weight raised to the 3/4 power (U.S. EPA, 2003b)

rats were provided by the authors (Ellis et al., 1984). Average body weights of 0.69, 0.65 and 0.52 kg for the males, and 0.41, 0.40 and 0.27 kg for the females, were derived from data provided in the text for the low-, middle- and high-dose groups, respectively. A default body weight (human) of 70 kg was used in the dose transformations.

A weight of evidence evaluation supports the conclusion that a determination of a mutagenic MOA for carcinogenicity cannot be made because there are insufficient data for determining the mutagenicity, or for defining an MOA.

In accordance with the U.S. EPA (2005b) guidelines, the LED_{10} (lower bound on dose estimated to produce a 10% increase in tumor incidence over background) was estimated from the incidence data using the U.S. EPA (2000) benchmark dose methodology (version 1.3.2), and a linear extrapolation to the origin was performed by dividing the LED_{10} into 0.1 (10%).

The LED_{10} value based on the male rat data (6.01 mg/kg-day) was selected as the point of departure for calculation of the oral slope factor, since this gives a slightly more health protective value than the LED_{10} based on the female rat data (**Table 3**). Linear extrapolation to the origin (0.1/ 6.01 mg/kg-day) results in a **human provisional oral slope factor of 1.7E-2 (mg/kg-day)$^{-1}$**.

Table 3. Multi-stage modeling results for hepatocellular tumors in male and female rats administered nitroglycerin in the diet*

Sex	BMD (ED_{10})[a]	BMDL (LED_{10})[a]	x^2 p-value	AIC
Male[b]	8.95	**6.01**	0.4549	62.134
Female[b]	10.88	7.39	0.7351	65.347

* Source: Ellis et al., 1984
[a] = mg nitroglycerin/kg-day
[b] = Restrict betas >=0, Degree of polynomial = 3

Provisional Inhalation Unit Risk

There are no human or animal inhalation data from which to derive a provisional inhalation unit risk for nitroglycerin.

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